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Torrey Botanical Club

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BULLETIN OF THE TORREY BOTANICAL CLUB
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THE EMBRYO OF JOUVEA PILOSA AS FURTHER EVIDENCE FOR THE FOLIAR NATURE OF THE COLEOPTILE[1]

JOHN R. REEDER

In a recent paper (Reeder 1953a) the writer reported that the embryo of *Streptochaeta spicata* possesses unique features which have a direct bearing on the homology of the coleoptile in the grass embryo. It was pointed out that in the embryo of this species the coleoptile possesses four well-developed bundles and the rudiment of a fifth. This fifth bundle, moreover, is median and on the side next to the scutellum. Furthermore, the coleoptile in the *Streptochaeta* embryo is not the usual closed stucture, but has free margins which overlap in the same manner as those of the embryonic foliage leaves within. Other workers (Kennedy 1899, Percival 1927, Avery 1928 and Jakovlev 1937) had previously reported coleoptiles with more than two bundles, but had not found a median bundle in any case. Since a grass leaf always possesses a well-developed midrib, its supposed absence in the coleoptile had been rather damaging to the interpretation of that organ as the homologue of a leaf. While it is true that in *Streptochaeta* the median bundle is rudimentary, the presence of this rudiment, together with the free and overlapping margins, suggests strongly that the coleoptile and the leaf are homologous structures.

Recently the writer has found that the embryo of *Jouvea* possesses the unusual features which he had first reported in *Streptochaeta,* although these two genera are quite unrelated. In the embryo of *Jouvea pilosa* (Presl) Scribn., the coleoptile, like that in *Streptochaeta,* is not a closed structure (fig. 1, C—F), but has free margins which overlap as do those of many embryonic foliage leaves. This figure also shows that there is a well-developed midrib which extends nearly to the tip of the organ. Serial transverse sections of the coleoptile reveal three equally well-developed vascular bundles. The median bundle is located opposite the free margins and next to the scutellum in exactly the position one would expect if the coleoptile were a leaf. This midrib is also evident as a strong vascular strand in a median sagittal section of the embryo (fig. 1, A).

Although the embryos of *Streptochaeta* and *Jouvea* are similar in the features which have a direct bearing on the question of the homology of the

[1] This research was supported in part by a grant from the National Science Foundation. I am grateful to the administration of Yale University for providing financial assistance by means of a Blanche Elizabeth MacLeish Billings Memorial Award. This made it possible for me to spend the summer of 1953 in Mexico where I was able to collect material for this study.

[THE BULLETIN for November–December 1955 (82: 419–535) was issued January 19, 1956.]

coleoptile, there are certain differences. In the former genus, the coleoptile possesses five vascular bundles, and the median bundle which is evident in only 2 or 3 serial sections cut at 10 μ near the base of the organ, is very weakly developed. In the coleoptile of *Jouvea,* on the other hand, only three bundles are present, but the median (and most significant) bundle is developed quite as strongly as the other two. In both genera, the coleoptile is open on the side away from the scutellum and the median bundle is situated opposite the open margins.

It seems highly significant that coleoptiles with median bundles and free and overlapping margins are found in genera as unrelated as *Strepto-*

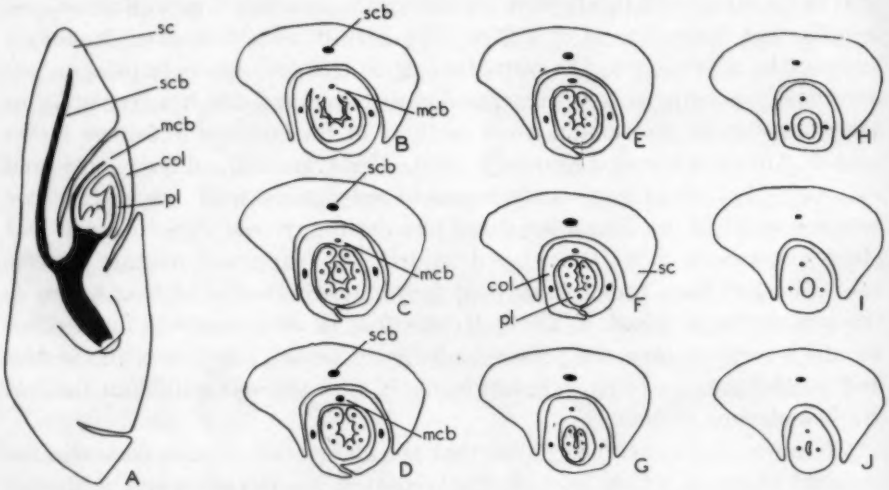

FIG. 1. Longitudinal and transverse sections of embryos of *Jouvea pilosa.* sc, scutellum; scb, scutellum bundle; mcb, median bundle of coleoptile; col, coleoptile; pl, first leaf of the plumule.—A, an approximately median sagittal section. Note especially the median coleoptile bundle which is well developed and extends nearly to the tip of the organ.—B—J, serial transverse sections through the region of the coleoptile from base to apex. Note the three coleoptile bundles, one of which is in the median position. Note also, especially in sections C—F, that the coleoptile is not a closed structure, but that the margins are free and overlap like those of many embryonic foliage leaves. These drawings were made with the aid of a microprojector.

chaeta and *Jouvea.* The form and vascularization of this organ suggest strongly that the coleoptile is, indeed, the homologue of a leaf. The fact that the median bundle in both genera is found on the same side as the scutellum would also suggest that the scuttellum is the first cotyledon, the second one being absent, and thus the coleoptile, the first leaf of the plumule, is the third leaf of the plant.

Since the taxonomic position of *Jouvea* has been a subject of some controversy over the years, it is perhaps worthwhile to give some attention to the relationships of this genus as suggested by a study of the embryo. The

genus comprises only two species: *J. straminea* Fourn. and *J. pilosa* (Presl) Scribn., both found on sandy seashores of the Pacific coast of Mexico and Central America. In general appearance these grasses somewhat resemble *Distichlis,* and like the members of that genus, they are dioecious. While the staminate spikelets present no problem in interpretation, the pistillate structure is rather complicated. The ultimate unit is a curved thorn-like structure in the sides of which the flowers are embedded in two rows in alternate arrangement. This structure was at first considered to be a spike, and Hackel (1887) placed *Jouvea* with the Hordeae. Scribner (1890) also considered the structure a spike, but in a later paper (1896) he suggested that it is probably a spikelet. Hitchcock (1930) refers to the structure as a spikelet, but continued to place the genus with the Hordeae. Weatherwax (1939), after a careful morphological study of both species, concluded that the pistillate structure is certainly a spikelet and suggested that the genus should be removed from the Hordeae and transferred to the Festuceae near *Distichlis* and *Uniola.* Prat (1933), who also studied both species, concluded on the basis of the anatomical structure of the leaves and epidermis that *Jouvea* is not allied to any member of the Festucoideae but that its affinities are with the Panicoideae. The present study of the embryo lends support to this view.

It has been demonstrated that there are striking differences between the embryos of members of the Panicoideae and the Festucoideae (Reeder 1953b). In the former group the lower part of the scutellum is free from the coleorhiza and the coleoptile is inserted well above the point of divergence of the scutellum bundle. In the Festucoideae, on the other hand, the lower part of the scutellum is fused to the coleorhiza, or is lacking, and the coleoptile is inserted at approximately the point of divergence of the scutellum bundle. As seen in longitudinal section, the embryo of *Jouvea pilosa* has the characteristics of the Panicoideae (fig. 1, A.). It is interesting to note that the embryos of *Distichlis* and *Uniola* are similar to those of *Jouvea,* and thus it appears that Weatherwax was correct in considering these genera to be rather closely related. To include them in the Festucoideae, however, is not consistent with the findings of cytological, anatomical, and embryo studies. Rather, these genera appear to be allied to the Chlorideae, which Prat, on the basis of his anatomical studies, considered to be a sub-group of the Panicoideae. Embryo studies tend to substantiate Prat's view. In the Chlorideae and allied genera, the embryo, while basically panicoid, differs in having always an epiblast.

SUMMARY

1. The embryo of *Jouvea pilosa* (Presl) Scribn. has been found to possess features similar to those previously described in *Streptochaeta spicata*

Schrad. ex Nees, and these features lend additional support to the view that the coleoptile in the grass embryo is the homologue of a leaf.

2. In both these species the coleoptile is not the usual closed structure, but the margins are free and overlap.

3. A median vascular bundle is present also which, while rather weak in *Streptochaeta,* is strongly developed in *Jouvea.*

4. On the basis of embryo structure, the affinities of *Jouvea* appear to be with the Panicoideae. This suggestion is in agreement with the view of Prat, who studied the anatomy of the leaf and epidermis.

5. The embryos of *Jouvea, Distichlis,* and *Uniola* are similar, which indicates that these genera are related, as suggested by Weatherwax. To place them in the Festucoideae, however, is not consistent with either the findings of Prat or the results of the present study.

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NOTES ON THE BIOLOGY OF MYRIOPHYLLUM SPICATUM L. IN A NEW JERSEY LAKE[1]

BERNARD C. PATTEN JR.

Myriophyllum spicatum L. is a large perennial water-milfoil (Haloragaceae) which has, with several other members of its genus, become a serious weed problem in numerous lakes and ponds of northern New Jersey. This study was conducted principally at Lake Musconetcong, a 329-acre shallow, alkaline, eutrophic impoundment which supports a flora including eighteen taxa of submergent phanerogams. Approximately 98 percent of this lake's underwater area is populated by *M. spicatum*. The work was supported by the New Jersey Department of Conservation and Economic Development whose officials required information upon which to base a life cycle approach to control because chemical techniques had previously proven of no value.

Ecology. The density of *M. spicatum* in a given location in Lake Musconetcong is determined primarily by substratum particle size, secondarily by depth of water (light) and temperature. Maximum density coincides with the distribution of fine organic ooze and diminishes to nearly total absence on sand. Depth is generally exclusive in excess of about seven feet. Other things equal, warmer temperature favors greater luxuriance.

The Myriophyllums are generally recognized as having definite affinities for alkaline situations. Often under highly calcareous conditions they precipitate an encrustation of marl (Hegi 1926). This occurs in the population of *M. spicatum* subsp. *exalbescens* in Gardner's Pond, Andover, New Jersey. In Lake Musconetcong the species is instrumental in maintaining a high pH. The daily pH cycle is so closely related to the physiological activities of these plants that in a 24-hour series of measurements at one site a fluctuation of 3.8 units was recorded, the maxima and minima correspondingly directly with photosynthetic activity. During the period of highest pH and maximum photosynthesis, bicarbonate alkalinity dropped concomitant with the appearance of several ppm. of carbonate indicating photosynthetic utilization of half-bound CO_2. *M. spicatum* is absent from the acid waters of the Coastal Plain in New Jersey, as it is also in Lake Musconetcong at local sites of low pH (ecotonal coves where a number of more acid-tolerant forms thrive).

Because of their abundance in the lake, aquatic plants are basic in its

[1] From a thesis completed in partial fulfillment of the requirements for the degree of Master of Science at Rutgers University under the supervision of Professors Edwin T. Moul and Murray F. Buell, to both of whom and to Mr. Thomas W. Haigh and his staff of the New Jersey Forests and Parks Section the author's gratitude is expressed.

productivity and eutrophication. The myriad organisms attached and otherwise associated with them are a food source for higher forms as *Aufwuchs* and, detached, as tychoplankton. The economic significance of *Myriophyllum* is emphasized by a study (Krecker 1939) which indicated this genus to be capable of supporting a far greater concentration of periphyton per unit length of stem than most other aquatic phanerogams because of the larger surface/volume ratio offered through its many finely dissected leaves.

Phenology. In 1953, earliest flowering was recorded on June 19 at Lake Musconetcong, but this comprised only a small number of inflorescences. However, at Lake Lackawanna only a few miles away first flowering was noted on May 29. This was somewhat more extensive but still constituted a fraction of the total population. Such early activity as this is not contiguous with the main flowering season which occurs later in the summer. During the first week of July the fruits of this pre-seasonal activity were examined and all proved to be inviable. Primordial inflorescences appeared on July 11 in a shallow cove on the lee side of Lake Musconetcong. Two days later these were abundant. Toward the beginning of August the plants of this area were in full bloom and producing fertile fruits. At the same time very little sexual activity was apparent in plants of most other areas. Gradually, flowering extended from the shallow, protected coves of the lee side out into deeper, more open water. The plants flower first, then, at the sites where they achieve their greatest luxuriance due to factors already mentioned.

On August 19, which approximated the peak of the flowering period for the lake as a whole, fifty-six plants were collected from a protected area (mean depth 122 cm.) and fifty-eight others from an open-water site (depth 163 cm.). The mean stem lengths of these series were, respectively, 140 cm. and 156 cm. Mainstems and laterals were counted and classified into one of the seven categories of table 1 on the basis of their termini. The table shows considerable dissimilarity between the two samples concerning the absence of any kind of terminating structure. This absence represents the cumulative effect of factors such as post-flowering auto-fragmentation, fragmentation

TABLE 1. *Percentage summary of stem and branch tips in various stages of activity on August 19, 1953. Series I was collected at a shallow, protected site; Series II in deeper, open water. Minimum temperature at site I averaged 7° F. more than that at site II during the summer.*

Stage of terminal activity	Series I		Series II	
No vegetative apex or inflorescence	39.76		57.43	
Vegetative apex only	4.42	(7.34)	9.64	(22.65)
Inflorescence: primordial	3.21	(5.33)	2.40	(5.64)
Inflorescence: stigmas only ripened	4.42	(7.34)	1.20	(2.82)
Inflorescence: anthers ripened	2.81	(4.66)	1.20	(2.82)
Inflorescence: fruiting	35.74	(59.33)	27.31	(64.18)
Fruiting, but male parts abscised	9.64	(16.00)	0.80	(1.88)

due to human activity, and stems remaining from previous flowering cycles. The plants of the open water show this more than those from the cove. The parenthetical figures in the table represent the percentage composition of only those stems which had terminal structures. They indicate that the population in the cove was more advanced through the flowering cycle on this date because fewer vegetative buds remained, almost twice as many stems were in some stage of flowering, and there was a greater incidence of fruiting. The prodigious proportions of the weed problem in Lake Musconetcong can be rather lucidly emphasized by pointing out that the inflorescences visible in the foreground of figure 1 represent only about two percent of all stems and branches present there, and those of the background producing what appears as a narrow, white band only seven percent.

By the middle of September, flowering is about ended and the fruiting spikes lay just beneath the surface of the water. In 1952 the last flowers were recorded on October 7. At that time, red and green vegetative buds were profuse, their color appearing to be a local phenomenon (which will be explained shortly). A month later most of the fruits were well-loosened from the deteriorating spikes and beginning to drop off. During this period the spikes as well as a considerable number of sterile members began fragmenting, accumulating in tangled masses on the windward shores. Axillary buds were produced at this time and extensive branch systems and adventitious rootlets developed by late November. This same adventitious activity occurred concurrently in the rooted segment of the population. Half or more of the year's seed crop was released by the end of November. Many achenes remained attached to the plants throughout the winter months and quite often were frozen into the ice. Fruits no longer remained by late February, and the number of freely-floating fragments was considerably diminished compared to early winter. That adventitious growth continued all winter was indicated by the abundant branching and general luxuriance characterizing the early vernal plants. In March, small, red, slime-covered and readily detached axillary buds were profuse. These dropped and germinated into small, independent plants by mid-April. In addition to this means of spring revegetation, sprouting from already existing rhizomes was also effective.

The seed stage. This phase of the life cycle has previously been discussed (Patten 1955) so that here it is desirable merely to summarize the material and so provide it context. Seed of *M. spicatum* does not tend to germinate soon after its development unless exposed to certain conditioning treatments (partial endocarp removal, scarification, freezing, drying and exposure to extremes of pH). The need for a period of after-ripening is implicit in the increased germinating capacity of year-old achenes compared to newly-developed ones. Delayed germination is effected by the pericarps which inhibit immediate activity. The conditioning treatments presumably act upon the

FIG. 1. Maximum blooming of *M. spicatum* on Lake Musconetcong, August 18, 1953.
FIG. 2. Habit of *M. spicatum* in flower illustrating the characteristic curvature of the stem just below the sexual axis.

pericarps to decrease their effectiveness in this capacity. The method of storage (in water, 5° C.) and experimental results suggest a minimum temperature below which germination does not proceed readily during the first few years (probably between 10–20° C.). Under natural conditions, then, it is likely that low winter temperatures and occasional freezing subdue germination, at the same time increasing after-ripening so that prompt germination is assured with spring warming. Similarly, the ability of seed to withstand desiccation and at the same time to become more susceptible to quick germination with the return of favorable conditions is clearly a protective modification against drying of a site and consequent decimation of the parent population.

There is no direct procedure for determining the magnitude of seed viability, but germination tests indicated high reproductive potential. Up to 85 percent germination was obtained with material from Lake Musconetcong, and Guppy (1897) achieved 89 percent in one test. Viability of a crop of seed probably extends over a number of years, perhaps ten or more. Guppy (1897) presented results from a six year experiment which indicated loss of viability after two, or at most three, years. The present tests, however, suggest that Guppy's achenes would have been more freely active for a longer period had they been subjected periodically to one or more of the conditioning treatments listed, which they were not.

Very little mortality of seed was indicated. Waterfowl, and probably other fauna as well, feed upon the achenes. To evaluate the rôle of fishes, sixty-eight digestive tracts of five species of teleosts netted in a heavily-seeded area during the peak of the fruiting season were examined. Only one achene was recovered from the tract of a yellow perch (*Perca flavescens*). From this it seems safe to infer that fishes account for little mortality of seed. In fact, the achene taken from the perch was situated at the lower end of the intestine immediately cephalad of the rectum suggesting that seeds ingested are not necessarily seeds digested. If the results with dilute acid and base solutions in the germination experiments can be taken as a criterion, it may be that after-ripening is enhanced by passage through the alimentary canals of animals.

The partial and complete removal of endocarps in one experiment provided an opportunity to observe something of the mechanical aspects of germination. One embryo with its surrounding testa was completely dissected out of its enveloping nutlet. Subsequent growth produced a swelling at the midpoint of the endosperm which threatened to rupture the testa, but eventually the radical emerged through the micropylar orifice. In contrast, embryos with only the micropylar half of their mesocarps removed germinated normally. In several instances when more than half was removed, bulging as above occurred. In one such case the testa ruptured at the mid-

point allowing the embryo to grow in an abnormal direction. Concurrent growth of this individual through the micropyle produced a bifurcate teratological specimen which soon died. From this series of observations it may be inferred that the stony part of each fruit is mechanically essential to proper germination, resisting an adventitious growth potential at all points except the micropyle.

Dispersal of seed to other regions proceeds by various means. The achenes, having a greater specific gravity than water, sink to the bottom when released. Since flow in the lake is very slight they are probably not carried far from the sites of deposition. However, fruit-containing spikes are capable of flotation once detached and so achieve wide dispersal in the drainage system. Hegi (1926) has noted that seeds caught in ice floes can also extend the range of the species downstream. During the autumn, large flocks of migrant waterfowl frequent Lake Musconetcong and feed extensively on achenes at the surface. Martin and Uhler (1939) and Martin, Zim

TABLE 2. *Percentage comparison of the relative lengths of epicotyls, hypocotyls and cotyledons of two series of seedlings on the fourth day following germination. The variates were all germinated within an hour of one another.*

Series	Stem	Rootlet	Cotyledons
Grown in tap water over soil	55.5	27.0	17.5
Grown in tap water only	24.8	48.7	26.5

and Nelson (1951) point out that achenes of *Myriophyllum* are utilized by ducks but are low grade foods. Guppy (1897) suggested that germination of seed may be increased by passage through the alimentary system of birds, a speculation which has already been given some tenability for fishes. Waterfowl, then, are probably the most important dispersal agents of fruits to other drainage systems; especially to the south, the direction of autumnal migration.

The seedling stage. Ecesis has been observed many times in the laboratory. As the radical emerges it pushes aside the micropylar plug, a small stopper-like structure of lignified cells inhabiting the space of the micropyle. Positive geotropism is exhibited immediately and the rootlet grows downward into the substrate. In the absence of soil, hypocotyl and cotyledon growth increase at the expense of epicotyl development (table 2), a response which would seemingly favor survival on sparse soil or where nutrient supply is suboptimal. Within a few days a lateral arises from the radical which in adult plants becomes the horizontal rhizome. Soon after the appearance of the radical the seedling frees itself from the nutlet. The subsequent period of a day or so sees rapid development of the cotyledons (usually two, but rarely with a third) which soon open outward from their originally ap-

pressed condition. Unlike the foliage leaves, the cotyledons are linear-lanceolate and undissected. Two small prophylls are next to develop. These first leaves may or may not grow to a maximum length of half a centimeter and in the process become dissected into two, three or four segments. With the development of the leaves of the second node, ecesis may be considered complete, for if this stage is not achieved within several weeks the cotyledons decline and the plant dies. Many seedlings grown in the laboratory failed to complete ecesis. None ever reached the stage of developing a second pair of foliage leaves until late spring of the first year. After this seed age was reached, however, most seedlings developed to and beyond this stage. Winne (1935) cited an observation similar to this to the effect that seedlings of *Trapa natans* germinated in January averaged 4.5 cm. of hypocotyl growth before the primary node appeared, but in May the average was only 1–2 cm. suggesting, perhaps, that after-ripening facilitates ecesis. The leaves of the second node of *M. spicatum* characteristically possess 4–6 divisions and, although no seedlings were grown beyond the third node, it would appear that the number of divisions increases progressively along the stem until the full parent complement is attained. It is not known where along the axis the verticillate condition develops. The early leaves are paired and opposite in two ranks.

Illumination was indicated to be critically limiting to seedling growth. Etiolation is pronounced in absence of light, but this response can be minimized by short periods of intermittent exposure to daylight. Shading from direct sunlight was shown to be sufficient cause for mortality.

Exhaustive searches underwater indicated that no seedlings are produced from year to year under the prevailing flooded conditions of Lake Musconetcong. Germinants would be prone to high mortality from both predation and intolerance to shading (by parent plants and perhaps even by suspended particles in the water). Sexual reproduction, therefore, is of only minor significance in maintaining the species in situations such as Lake Musconetcong.

The juvenile stage. This stage can be considered as the period from the completion of ecesis to the inception of floral apices. In the instance of a vegetative fragment, it is understood to begin the moment separation occurs in which case ecesis and growth must be effected before flowers will be produced.

The mature plant rarely comprises a single stem, there being usually several to perhaps a dozen attached to a single rhizome. Hundreds of long, silvery, fibrous roots are also attached to the rhizome. These are all adventitious in origin, their primordia having arisen in the pericycle (Hegi 1926). The primary root serves only for initial attachment of the seedling to the substratum, after which it deteriorates.

Mechanical support of the growing stems is provided by the many finely dissected leaves. The lower leaves are either caducous or shaded-out since mature stems are characteristically devoid of leaves at the lower nodes.

The reproductive stage, sexual. The inflorescence of *M. spicatum* may be described briefly by condensing the treatments provided by Vaucher (1841), Knupp (1911), Hegi (1926) and Fernald (1950). It is a terminal, naked, interrupted spike, indeterminate in development and often very red. The flowers are usually functionally monoecious, but they may occasionally be polygamous. Vestiges of the parts of the suppressed sex remain. The upper flowers on a spike are staminate and the lower pistillate, the transition between appearing abrupt, but being actually graded as revealed by histological preparations (Knupp 1911). The entire spike may sometimes be either staminate- or pistillate-dioecious. This is considered to be a vestigial condition of a completely hermaphroditic phylogenetic origin of the group (Knupp 1911 and Hegi 1926). Schindler (quoted by Hegi 1926) believed the surrounding aqueous medium to be the direct cause of repression of the male sex in the lower flowers. Each flower is sessile or nearly sessile in the axile of a strong bract. Two more delicate bracteoles are also located at the base of each flower. These three bracts entirely enclose and protect the young flower. They gradually open out as the spike elongates and the flowers mature. The flowers are verticillate in fours, each whorl being rotated about forty-five degrees from the ones adjacent so that the whorls are in two ranks. This rotation produces the spiral arrangement characteristic of the spikes before elongation.

The first suggestion of flowering in the field is the appearance of small, green, compressed (telescoped) spikes at the stem apices, covered at least partially by the last of the leaves produced by the vegetative apex. These leaves are deeply pigmented red. It has been stated that the red coloration of foliage appeared in general to be a localized phenomenon. While in the bud the leaves are covered with multicellular caducous trichomes which arise basipetally (as do the leaves themselves) and secrete a glucosidic slime whose supposed rôle is protection. This highly refractile, readily oxidizable slime turns bright cherry red when treated with vanillin or hydrochloric acid (Raciborski 1893 and Proscher 1895). Proscher had named this product *hydroxymyriophyllin*. It is a pH-sensitive anthocyanidin and indicates in the lake local sites of comparatively high acidity.

The flowering cycle from the first early stage described above entails, briefly, an elongation of the spike axis, elevation of the spike out of the water, pollination and resubmergence. No account of the mechanics of spike elevation appears in the literature although Vaucher (1841) reports that spike axes become thicker than the normal vegetative stem and that the blooms occur just above the water surface regardless of depth. As the spikes

elongate, the stem internodes just beneath also elongate allowing the leaves at the base of the spike to float the latter just at the water surface. The spike axis becomes nearly twice as thick as the normal stem and very rigid. This combination makes it very succulent and floatable. Still, the leaves are not sufficient support to force the growing spike vertically into the atmosphere. Instead, the floating spike curves just below the pistillate flowers. This curvature is in such a direction in relation to the whole plant that the tip of the spike is formed to emerge as long as the flotation mechanism is functioning properly. The curvature is apparent in figure 2. The inferior, or convex, side of the bend becomes very rigid so that the curved position is firmly maintained. So effective is this elevation-flotation mechanism that even inflorescences accidentally fragmented by human activity maintain their ability to keep the flowers above water as long as there is at least half a foot or so of stem length beneath to provide counterbalance.

After the spikes emerge they exhibit protogynous dichogamy, the stigmas ripening well in advance of the stamens, thereby favoring cross-pollination. Soon after, the deciduous petals abscise forecasting the ripening of the stamens. At this time they cover the water surface in profusion and wind currents deposit them around floating debris and on the windward shores.

Pollination occurs when the spikes project out of the water, dichogamy preventing self-pollination. The mode of pollination has been the subject of diverse opinions. Arber (1920) states that "... a certain number of aquatic plants appear to have given up insect-pollination and taken to anemophily, often with concomitant simplification of the flower." She alluded that *Myriophyllum* was one such genus as evidenced by the structural adaptation of long anthers swinging on flexible filaments. Hegi (1926) also favored wind-pollination. Ludwig (1881) cited wind-pollination although he notes that whole populations of *M. verticillatum* have been observed bearing flowers underwater. Knuth (1908) reported that Ludwig cited hydrophily as well as anemophily for *M. spicatum*. Hegi (1926) also mentions hydrophily in *M. verticillatum*. Vaucher (1841), apparently overlooking the protogynous nature of *M. spicatum*, stated that pollen falls out of the anthers directly onto the stigmas below to effect fertilization. Knupp (1911) explores the problem in some detail and concludes in favor of hydrophily for *M. spicatum*.

Auto-pollination in *M. spicatum* would be generally ruled out with the following possible exception. A single plant usually possesses a number of flowering stems each of which has lateral branches which also flower. It happens that the main stems bloom before the laterals so that pollen from the central spike can be ripe at the same time the lateral pistils are receptive. Even here, though, the chances for cross-pollination would still be greater by virtue of the greater abundance of pollen freed from other sources.

In spite of the views of Knupp and Knuth, hydrophilous pollination appears not to occur in *M. spicatum* in Lake Musconetcong. This is substantiated by the observation that the lowermost carpels on some inflorescences are often submerged, at no time being emersed. As during pre-seasonal blooming when carpels abort because of a paucity of pollen, such submerged carpels have always been observed to abort instead of developing into mature achenes. Indeed, many spikes lean at an angle away from the perpendicular out of the water causing submergence of some of the carpels of a whorl while the others remain exposed to the atmosphere. The abortion of these submerged carpels is quite in contrast to the successful maturation of the emersed ones at the same node. The evidence points to wind-pollination with entomophily perhaps assuming some significance. This is supported by the fact that the glazed, light pollen is quickly reduced to dust upon dehiscence of the anthers.

Following pollination the staminate parts of the spikes decline, often abscising completely, and the pistillate portion with the staminate remnant disappears beneath the surface with a concomitant decrease in the thickness of the spike axis. At this time, the lowermost pistillate bracts often develop basipetally into the normal submergent type of leaf, acquiring as many as five or six pairs of divisions. This suggests a close affinity of this species with *M. verticillatum*, which differs primarily in the nature of these bracts. And at least several workers have indicated a belief that *M. spicatum* is an offshoot of a vast and variable *M. verticillatum* complex which, perhaps, should not be considered a definitive species (Gmelin, Perrot 1900, Patten 1954). The leaves of the post-flowering stem buoy up the fruiting spike just under the water surface, and the fruits remain attached for a considerable time undergoing maturation. During this period the spikes slowly disintegrate until they are sufficiently rotted for the achenes to drop off passively with the aid of wave action.

The reproductive stage, asexual. Since sexual reproduction of *Myriophyllum* in Lake Musconetcong is inconsequential under the current ecological regimen, virtually all new plant material is produced by asexual means. One of the most significant attributes of aquatic plants in general, in spite of taxonomic diversity, is the ability to reproduce by vegetative fragmentation. Hegi (1926) states that many populations of *M. spicatum* are always sterile or only rarely produce flowers, and this can be verified for several other species as well in many ponds of northern New Jersey. An excised or an abscised stem will vigorously sprout adventitious roots, bud profusely, and increase in size. Such fragments float at first, but ultimately sink to a level where the roots are able to grasp the substratum.

The effectiveness of fragmentation was gauged by denuding a 1×4 meter quadrat in a heavily vegetated area on July 11, 1953. A buffer zone one

meter in width was also cleared. Fifteen rooted individuals were established in the quadrat by July 31. These were about a decimeter in height, but several were larger. On August 12, thirty-four plants were present, mostly 2–3 dm. tall. Fifty-eight individuals were recorded on August 18, and better than eighty on September 2. At this time the buffer zone was seen to contain considerably more vegetation than the quadrat indicating the process to be one of encroachment. By the following April, the quadrat and its buffer zone were indistinguishable vegetationally from the surrounding area. Not a single specimen of another species (seventeen other phanerogams) became established.

During late winter and early spring the plants develop a profusion of small axillary buds which differ from normal vegetative buds in their size and the ease with which they may be detached. The abscision of these structures and of other buds and stems throughout the year is effected by the formation of a spurious separation zone of partially lignified cells and not, as Hegi (1926) has suggested, by a rotting away at the base as with the sexual spikes. The nature of these small buds is dubious. Many species of *Myriophyllum* develop large and prominent winter buds known as turions (Arber 1920, Hegi 1926) or hibernaculae (Stover 1951). These are perennating in nature. According to Arber, turions were for many years considered as adaptations for overwintering. Goebel (1891–1893) discovered that turion formation in *M. verticillatum* is the result of unfavorable habitat conditions. Glück (1906) has further shown that plants grown under the intensely competitive conditions of a crowded vessel will develop winter buds (so-called) in the spring. Apparently this is the prevailing condition in most of the individuals inhabiting the dense population areas of Lake Musconetcong. Luxuriant specimens of the genus growing in water in warm situations are known to vegetate throughout the winter without developing winter buds. Arber (1920) states that under normal conditions it is probably the lowering of water temperature in winter which induces turion formation. Glück has shown in addition that continued growth of a stem axis which did not produce an inflorescence will result in the production of a turion. *M. spicatum* has been several times stated not to produce turions (Hegi 1926, Hultén 1947). Turions are generally large structures, up to several centimeters long, and they are prominent in New Jersey material of *M. verticillatum* and *M. spicatum* subsp. *exalbescens*. Also, they are characteristically terminal structures and not axillary. So morphologically the small spring buds of the Lake Musconetcong material are not turions. But physiologically, they may be so-considered. Detached, they germinate into independent young plants and so are responsible for much of the vernal revegetation which occurs. Apparently, here is a case of intermediacy between the turionate and aturionate condition in the genus.

Dispersal of the species by vegetative means is probably more successful than by sexual means. Fragments which float downstream are more likely to effect ecesis than seedlings. Even extension to other drainage systems is not an exclusive function of the sexual cycle. Hegi (1926) has stated that turions are able to adhere to the feet of waterfowl because of their glucosidic envelope. Thus the small, slime-covered buds of spring in Lake Musconetcong may represent just as effective a means of northward dispersal at this season as seed does to the south in the fall.

Sprouting from rhizomes is an additional mechanism for revegetation in the spring, and the effectiveness of this type of reproduction has been observed in mid-April at many sites which had been fairly devoid of vegetation earlier. The fact that sprouts were responsible for this growth was easily determined by collecting a number of plants and comparing the size of the rhizome with that of the new growth.

SUMMARY

1. This study describes the life cycle of *Myriophyllum spicatum* L., a serious weed control problem at Lake Musconetcong, New Jersey.

2. Density is determined primarily by substratum particle size and secondarily by depth (light) and temperature (ooze, shallowness and warmth favoring luxuriance). Affinities are for alkaline situations; high acidity is limiting. The species is basic in the economy of Lake Musconetcong because of its abundance. It determines the daily pH cycle and aids in maintaining a high pH. It provides support for the organisms which comprise the base of the food chain: *Aufwuchs* and tychoplankton.

3. *M. spicatum* vegetates perennially and flowers during late summer. Pre-seasonal blooming occurs to some extent at the beginning of the summer, but viable fruits are not reproduced. Flowering begins in the shallow, protected coves and extends out into open water as the season progresses. About 57 percent of all stems in the shallow areas and 33 percent in open water flower annually. The fruits drop from the plants in the several months following flowering. Growth and reproduction by fragmentation occur throughout the winter months. Toward early spring, small axillary buds are produced which abscise and germinate into independent plants. There is also considerable sprouting in spring from rhizomes. This and the new growth from small buds are the most significant means of vernal revegetation.

4. Germination is delayed, but a number of conditioning treatments including freezing and drying induce fairly prompt activity. The stony endocarps inhibit germination, and the conditioning treatments presumably decrease their effectiveness in this capacity. Accidental freezing or drying of seed in nature increases the capacity to germinate with the return of

favorable conditions. Generally, at least 80–90 percent of achenes produced are viable, and they may remain so for a decade or more. There are very few seed predators. Evidence suggested that achenes ingested by fishes or waterfowl would become more viable instead of being digested. The stony endocarp is essential to the proper germination of the embryo, serving to force the radical through the micropyle. Dispersal of seed within a drainage system occurs by normal downstream carriage of single achenes, and movement of achenes still attached to sexual spikes or frozen into ice. Dispersal to other drainages proceeds in the digestive tracts of waterfowl.

5. Seedlings are very vulnerable to predation and shading. Also, they must effect rapid ecesis. A response favoring the latter is the increased growth of hypocotyl and cotyledons at the expense of epicotyl development in the absence of soil. This indicates considerable plasticity regarding adaptability.

6. The primary root of the seedling serves only for initial attachment following which it aborts. The definitive root system is developed adventitiously. Mechanical support of the stem is provided by the leaves (although the stems themselves are about the same specific gravity as water). The lower leaves are either naturally caducous or shaded out as the plants mature.

7. Sexual reproduction is not significant in the present ecology of Lake Musconetcong in producing new plant material. But continuance of the species is assured by the presence of these very tolerant propagules. The sexual spike emerges from the water by a combination of factors: increased floatability of the spike produced by its swelling, curvature at the base of the spike in conjunction with the counter-thrust of the stem beneath, and increased rigidity of the spike allowing it to remain erected into the atmosphere. Self-pollination is limited by protogynous dichogamy. Pollination is aerial, somewhat entomophilous, but mostly anemophilous.

8. The production of all new material of *M. spicatum* in Lake Musconetcong occurs asexually by fragmentation and abscission of vegetative parts (including the small, turion-like buds of spring), and by adventitious sprouting from rhizomes. Even dispersal of the species to other drainage systems is partially achieved in this stage by the adherence of the small, slime-covered spring buds to the feet and plumage of birds. Most dispersal within the drainage is achieved by the downstream movement of floating fragments.

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THE SPECIAL MODE OF DEHISCENCE OF ANTHERS OF POLYGALA AND ITS SIGNIFICANCE IN AUTOGAMY

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The family Polygalaceae has been variously placed by different systematists. Chodat (quoted in Rendle 1952) describes it as "a very natural family, not closely allied with any others." Anthers of this family usually open by terminal pores and rarely by slits. *Polygala* is the largest genus of the family and anthers of the following four species have been investigated by me: *P. persicariaefolia DC.*, *P. chinensis* L., *P. triphylla* Buch-Ham., *P. triphylla* Buch-Ham. var. *glaucescens* Wall., and *P. abyssinica* R. Br.

Materials and methods. *P. persicariaefolia* and *P. triphylla* were collected from Chakrata in the lower Himalayas, *P. abyssinica* from Nainital also in the lower Himalayas and *P. chinensis* from Mysore in peninsular India. Flowers of different ages were fixed in formalin-acetic-alcohol. Gross features of flower and anthers were studied from material dissected and mounted in lactophenol. Histological features were studied from microtome sections of material imbedded in paraffin and cut at thicknesses varying from 8–12 μ. A combination of Heidenhain's haematoxylin and eosin in clove oil was used for staining.

The flower and pollination. The flowers are zygomorphic and bear a remarkable superficial resemblance to those of the Papilionatae. They conform to the formula K5, C3 A8, *G* (2). The inner lateral sepals are large, usually petaloid and wing-like. The lower median petal forms a keel enclosing the essential organs. In *P. persicariaefolia*, *P. chinensis* and *P. abyssinica* this petal is dorsally crested with a brush-like appendage. In *P. triphylla* where the flowers are much smaller, such an appendage is wanting but the keel has inflated terminal lobes. The lateral sepals recall the alae and the median petal the vexillum of a papilionaceous flower. The stamens are united below in a split sheath which is adnate at the base with the corolla.

As in the Papilionatae, the essential organs, which are enclosed in the keel, emerge when the latter is depressed by bees and other visiting insects. However, according to Chodat, the stigmas of *Polygala* appear to be adapted also for self pollination as they form a pocket into which the anthers open directly. Where such a pocket is absent, the anthers attach themselves directly to the stigmatic papillae.

Structure of the anther. Figures 1–6 show the morphology of the anther in the different species investigated. The anther is nearly oblong or pear-

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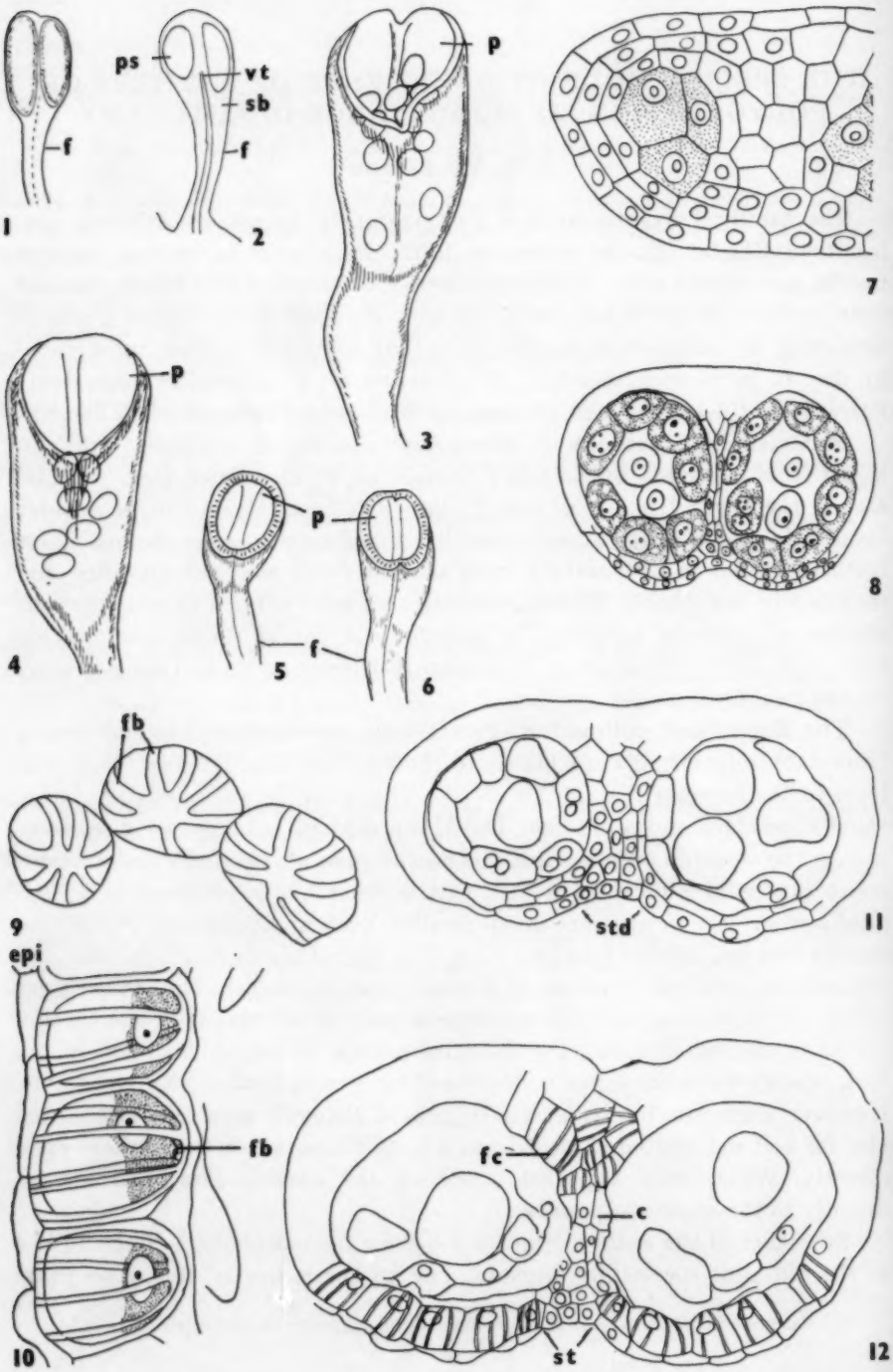
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shaped and its sterile tapering base merges insensibly with the filament. In all cases, the anther opens by a single large pore which extends nearly half way down its ventral side.

The vascular bundle of the filament ascends only up to the basal part of the connective and remains unbranched (fig. 2).

As a rule, there are only two microsporangia. The anther wall differentiates into the epidermis, hypodermis, an ephemeral middle layer and a tapetum of 1–2 nucleate cells (figs. 7 and 8). In later stages, the hypodermal cells enlarge and develop typical fibrous thickenings which originate from a common point on the inner tangential wall of the hypodermal cell. They run along its radial walls and terminate on its outer tangential wall beneath the epidermis (figs. 9 and 10).

A single stomium is differentiated on the ventral side of the upper part of the anther between the two pollen sacs. It originates as a shallow depression in the anther wall (fig. 11). In this region the epidermis, hypodermis, and the subjacent part of the connective tissue between the pollen sacs remain small and unthickened, so that the fibrous hypodermis as also the general epidermis, are discontinuous in the stomial area (fig. 12).

The stomium is more or less confined to the upper part of the anther. In the lower part, the general epidermis and the fibrous hypodermis continue undepressed and uninterrupted in the region where a stomium should normally have existed (fig. 13). In *P. persicariaefolia* the connective of this region possesses a transverse plate of small-celled subhypodermal tissue (fig. 13, *r*). This tissue connects the two pollen sacs laterally and tapers down to a narrow strip in the sterile base of the anther. In *P. chinensis* and *P. triphylla* such a tissue is absent.

A considerable part of the connective consists of enlarged cells which develop secondary fibrous thickenings similar to those in the hypodermal cells (figs. 12 and 13).

Dehiscence of the anther. Prior to dehiscence the pollen sacs become laterally confluent due to a dissolution of the unthickened part of the connective. Figure 14 shows an anther of *P. triphylla*, which is about to dehisce. The stomial part (*ds*) is covered only by the epidermis. The mechanical force developed by the shrinking fibrous cells effectively brings about not

FIGS. 1–12. (c, unthickened part of the connective; *epi*, epidermis; *f*, filament; *fb*, fibrous thickening; *fc*, fibrous part of connective; *p*, pore; *ps*, pollen sac; *sb*, sterile base of anther; *st*, stomium; *std*, stomial depression; *vt*, vascular trace). FIG. 1. Young stamen of *P. triphylla*. × 85. FIG. 2. L. s. young stamen of *P. persicariaefolia*. × 45. FIGS. 3–6. Open anthers of *P. chinensis* (fig. 3), *P. abyssinica* (fig. 4) and *P. triphylla* (figs. 5, 6) × 85. FIG. 7. Part of t. s. young anther of *P. persicariaefolia*. × 850. FIG. 8. T. s. young anther of *P. chinensis*. × 450. Fig. 9. Fibrous hypodermal cells of *P. triphylla* viewed from their inner sides. × 850. FIG. 10. Part of old anther wall of *P. persicariaefolia*. × 850. FIGS. 11, 12. T. s. young and old anthers respectively of *P. persicariaefolia* in the upper dehiscent region. × 450.

only the rupture of the weakened stomium but also spreads apart the split edges of the anther wall so as to result in the formation of a large pore which is roughly oval. The pore remains circumscribed to the upper half of the anther. Where no stomium existed before, the anther wall fails to split. Figures 15 and 16 show the open anther of *P. persicariaefolia* in the dehisced and undehisced regions respectively.

Abnormal anthers. Occasionally some anthers of *P. persicariaefolia* show 3 or even 4 pollen sacs (microsporangia). Here, the additional pollen sacs extend only for a short length of the anther and are invariably situated on its ventral side. Figures 17 to 20 are cross sections of an unopened anther containing 3 pollen sacs, and figures 21 to 24 are of a dehisced anther which originally contained 4 pollen sacs now fused in pairs. Such abnormal anthers dehisce like the normal ones.

Autogamy of the flowers. Dissections of flower buds of different ages of all the four species revealed the prevalence of autogamy. The essential organs remain enclosed in the boat-shaped keel petal (fig. 25). The form of the stigma varies from species to species. In *P. abyssinica* it is funnel-shaped (fig. 26), in *P. triphylla* it is oblique and fan-shaped (fig. 27), and in *P. chinensis* it is flattened and inflexed at the tip recalling the expanded hood of a snake (fig. 28). In all these species the stigma possesses a single papillate receptive lobe. But in *P. persicariaefolia* there are two such lobes flanking a pocket-like depression in the oblique stigma (fig. 29).

The style is longer than the stamens. At first it is nearly straight, but with further elongation, it becomes strongly curved parallel to the base of the keel (figs. 30–33). As a result of such curvature the stigma comes to lie close to the anthers which shed their pollen over it (figs. 34–36).

In *P. abyssinica* the receptive lobe of the stigma secretes a greenish glutinous substance which accumulates as a ball (fig. 26, *gl*) and causes some of the anthers to stick to the papillae long before opening (figs. 37 and 38). When dehiscence does occur, the pollen is emptied straight on to the papillate lobe of the stigma (fig. 39).

There was no indication of the occurrence of cross pollination in any of the open flowers examined, and the pollen lying around the stigma always appeared to belong to anthers of the same flower.

Discussion. The restricted dehiscence of the anther in this genus is as-

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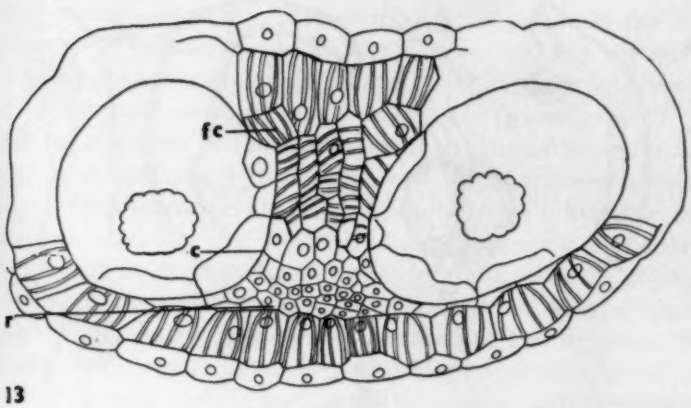
FIGS. 13–24. (*c*, unthickened part of the connective; *ds*, line of dehiscence; *fc*, fibrous part of connective; *fps*, fused pollen sacs; *p*, pore; *r*, small-celled tissue of connective). FIG. 13. T. s. old anther of *P. persicariaefolia* in the indehiscent region. ×450. FIG. 14. T. s. old anther of *P. triphylla* which is about to dehisce. ×400. FIGS. 15, 16. T. s. mature anther of *P. persicariaefolia* in the upper dehisced region and the lower undehisced part respectively. ×400. FIGS. 17–20. Serial transections of abnormal anther of *P. persicariaefolia* with three pollen sacs. ×85. FIGS. 21–24. Serial transections of dehisced abnormal anther of the same species that had four pollen sacs which are now confluent. ×85.



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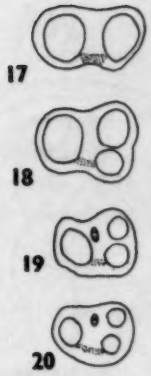

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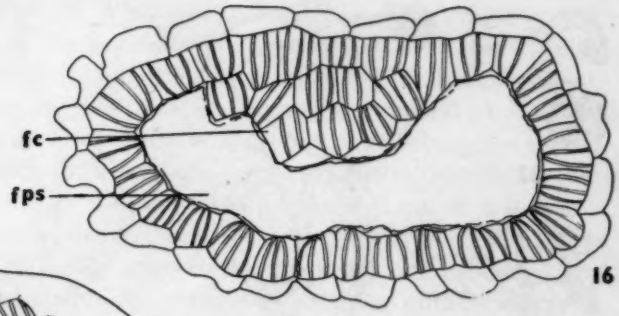

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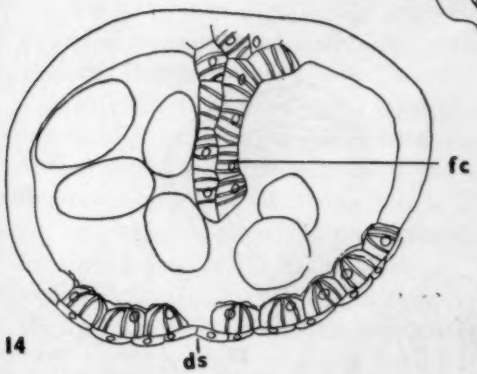

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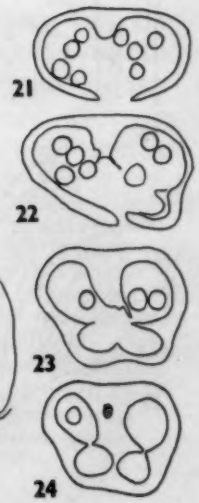

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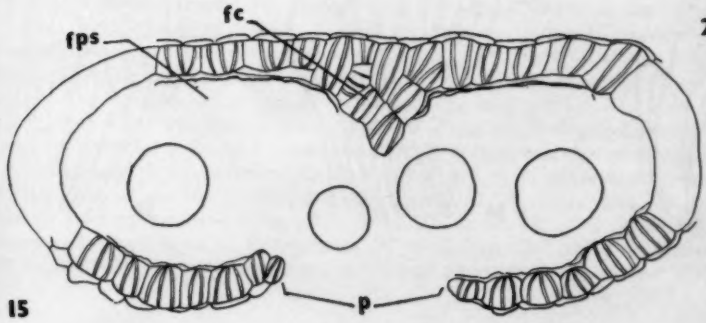

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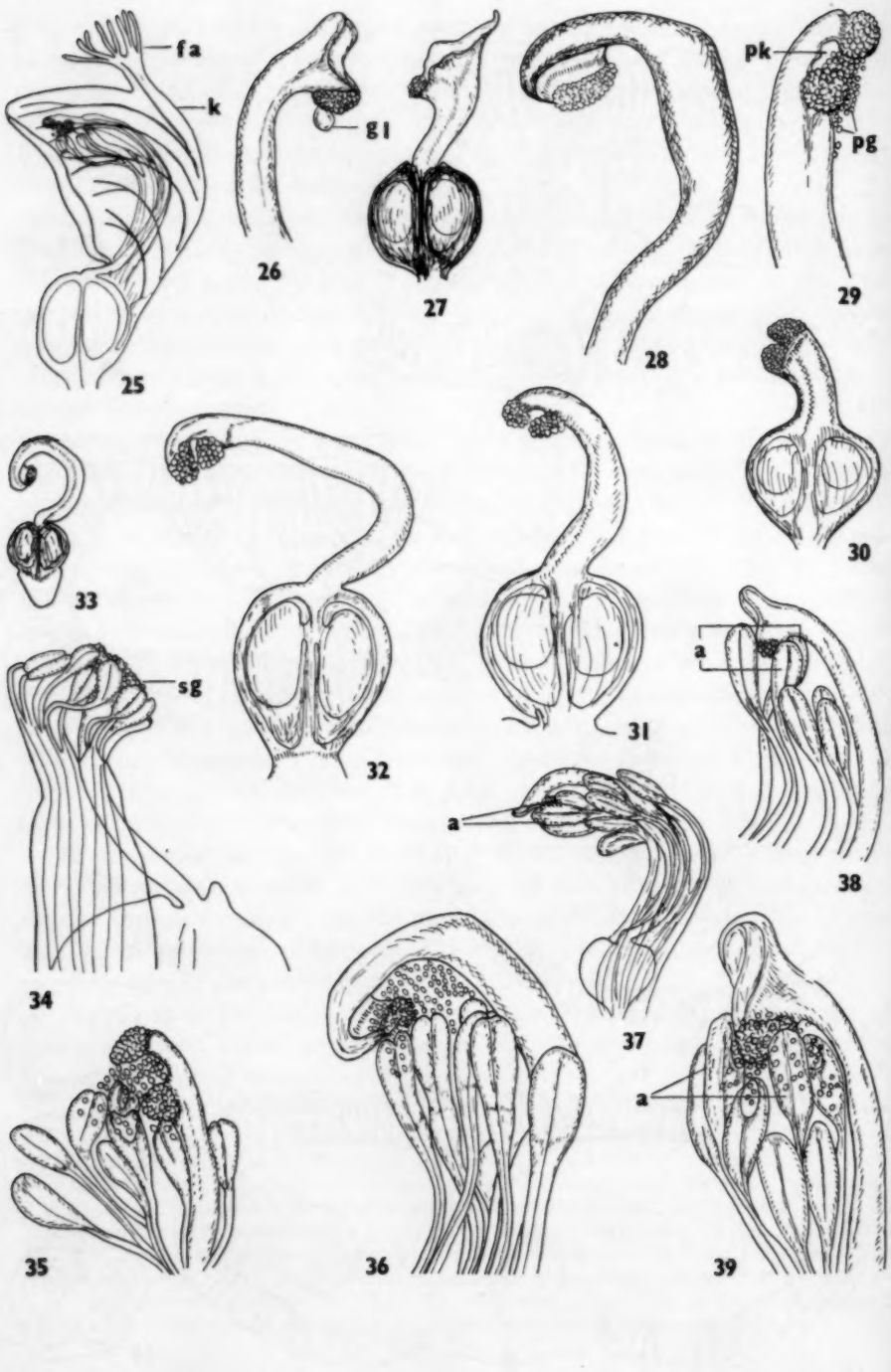
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sociated with an abbreviated stomial development in the anther wall. The stomium which develops only in the upper part of the anther, consists of unthickened and unenlarged cells of the epidermis, hypodermis and the subjacent unthickened part of the connective. Development of such a stomial area fails to occur in the lower part of the anther where, therefore, the general epidermis and hypodermis are continuous and unweakened by thin areas. The fibrous mechanism of the anther wall, however, is comparable to that prevalent in a typical longitudinally dehiscing angiospermic anther and does not show any trace whatever of localization in the dehiscent upper part of the anther alone. The mechanism is, however, very effective in that it not only causes the rupture of the stomium but also broadens the opening into a large gaping pore by widely spreading the split valves of the anther wall.

While most anthers have only two microsporangia, additional sporangia may be present in some anthers. Whenever such additional sporangia occur, they are always situated on the ventral side of the anther. It is clear, therefore, that the prevalent bilocular anther of *Polygala* is derived from an original quadrilocular condition by a suppression of the ventral sacs of the two thecae. The two pollen sacs that are now present thus belong not to the same theca but to two different thecae, and the sterile tissue that intervenes between them is homologous to the connective region and not to a septum. As to the mode of dehiscence, the abnormal anthers open poricidally in the same manner as the usual bilocular ones. From this it may be concluded that the poricidal mechanism existed in the genus even before its anthers attained the present bilocular state.

Autogamy is of regular occurrence in all the four species investigated. A curving of the long style brings the receptive stigma in the vicinity of or in actual contact with the anthers of the same flower. Each species has its own characteristic type of stigma, but in all of them there is some sort of a pocket into which the anthers discharge their pollen through the pores. In *P. abyssinica,* some of the anthers actually get stuck to the glutinous secretions of the stigmatic papillae, and they remain so until anthesis.

The apical poricidal dehiscence seems very significant when considered in

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FIGS. 25–39. Autogamy. (a, anthers that are actually fixed to the stigmatic papillae; *fa,* fimbriated appendage of keel; *gl,* ball of glutinous stigmatic secretion; *k,* keel petal enclosing the essential organs; *pg,* pollen grains; *pk,* pocket between the two stigmatic lobes; *sg,* stigma). FIG. 25. Flower of *P. persicariaefolia* with petals and all sepals removed to show the essential organs enclosed in the keel petal. × 10. FIG. 26. Stigma and part of style of *P. abyssinica.* × 27. FIG. 27. Pistil of *P. triphylla.* × 27. FIGS. 28, 29. Stigma and part of style of *P. chinensis and P. persicariaefolia* respectively. × 30. FIGS. 30–32. Pistils of *P. persicariaefolia* at three stages of development to show lengthening and curvature of the style. FIG. 30. × 30. FIGS. 31, 32. × 27. FIG. 33. Old pistil of *P. chinensis* showing incurved style. × 15. FIGS. 34–36. Shedding of pollen from the dehisced anthers on to the stigma in *P. triphylla, P. persicariaefolia* and *P. chinensis* respectively. × 30. FIGS. 37–39. Progressive stages in the autogamy of *P. abyssinica.* × 15.

relation to the autogamous pollination of the flowers in this genus (see figs. 34–39). Had pollen discharge occurred at the base of the anther, such pollen would be diffused in regions not readily accessible to the stigma. The subapical ventral pore ensures that the pollen is discharged only at the tip of the anther, a part which is nearest to or in actual physical contact with the stigma of the flower and thus adds precision to the process of autogamy.

SUMMARY

1. Anthers of all the four species of *Polygala* investigated here, are normally bilocular and contain only two microsporangia. Though the anthers differ in size and form, they are all alike in structure and dehiscence which occurs by a single large subapical pore on the ventral side.

2. The delicate anther wall differentiates into the epidermis, hypodermis, an ephemeral middle layer and the tapetum.

3. The hypodermis acquires secondary fibrous thickenings which are characteristically disposed on the cell walls.

4. Besides the hypodermis, cells of part of the connective also develop similar fibrous thickenings.

5. Prior to dehiscence, the pollen sacs become laterally confluent along their entire length by the breakdown of the intervening unthickened part of the connective.

6. The restricted poricidal dehiscence is primarily due to the abbreviated stomium and the fibrous mechanism does not show any localization in the dehiscent region.

7. From a study of abnormal anthers it is concluded that originally, anthers of this genus, were probably quadrilocular and with four microsporangia as in typical angiospermic anthers. The prevailing bilocular condition is the result of suppression of the two sporangia situated on the ventral side of the anther.

8. Autogamy is of regular occurrence and it is shown that the release of pollen through the apical pores of the anthers, is of special advantage for this process.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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THE GROWTH OF BARLEY EMBRYOS ON COCONUT MILK MEDIA[1]

KNUT J. NORSTOG

Coconut milk has been used with success in the culture of very small embryos of a number of plant species, notably of *Datura* (Van Overbeek, Conklin & Blakeslee 1941). However, it has not given comparable results with embryos of the grasses. Haagen-Smith, Siu & Wilson (1945) used coconut milk in culturing young maize embryos but found it was not beneficial. Ziebur and Brink (1951) report little success with coconut milk when used in concentrations of 20 to 50 per cent to culture immature barley embryos. In the present study, very small embryos of Moore Barley were cultured in order to study the effects of certain substances on embryogenesis. A

TABLE 1. *The growth of barley embryos* in vitro.

Medium	No. of embryos	Length of embryos		Culture period	Appearance
		initial	*mean final*		
A[1]	38	0.8 mm.	1.3 mm.	7 days	callused
B[2]	43	0.8	1.8	7	callused
C[3]	29	0.8	2.3	7	normal
A	20	0.5–0.6	1.2	7–9	callused
C	33	0.4–0.6	1.6	7–14	normal
C	5	0.3	1.6	14	normal

[1] White's medium.
[2] White's medium plus 20 per cent coconut milk.
[3] White's medium plus 90 per cent coconut milk.

medium containing 90 per cent coconut milk was found to stimulate the growth of even the smallest embryos cultured.

The embryos were aseptically removed from seeds, measured with an ocular micrometer and placed on nutrient agar. A modified White's solution (White 1943) was used as the basic medium. Coconut milk, when used as a supplement to this medium, was sterilized by Seitz filtration except for a few cultures made with a medium containing autoclaved coconut milk. The cultures were kept in darkness at room temperature for periods ranging up to thirty days.

Embryos cultured on White's agar or with 20 per cent coconut milk failed to develop properly. Their growth was mainly by cellular enlargement. Initiation and growth of root and leaf primordia were suppressed

[1] Part of a Ph.D thesis submitted to the Horace H. Rackham School of Graduate Studies, University of Michigan. Much of the work was completed during the tenure of a fellowship granted by the National Science Foundation, Washington, D. C.

and the embryos became callused and watery looking. However, on the 90 per cent coconut milk medium embryos attained the ivory color and firm

FIG. 1. A barley seedling produced from an 0.15 mm. embryo cultured 30 days on a medium containing 90 per cent coconut milk.

texture characteristics of normal development. Root and leaf primordia continued to be formed and in some instances came to equal (though not to

exceed) the number present in the ripe seed. Seedlings obtained from these embryos were strong and continued to evolve leaves and roots as long as they were kept in culture.

Four embryos, ranging in length from 0.15 to 0.2 mm., were grown on 90 per cent coconut milk. For a time all four grew as small balls of undifferentiated tissue. Later two of these produced leaves and roots. The smallest embryo eventually became a seedling which consisted of two coleoptiles, two leafy shoots and ten roots (fig. 1). The differentiation and organization observed in this embryo may have occurred through regeneration from the small bit of callus that first appeared. Supernumerary organs are reported to occur in the culture of small *Datura* embryos (Van Overbeek, Conklin & Blakeslee 1941) and perhaps are to be expected similarly in barley.

The effect of autoclaving on the 90 per cent coconut milk medium was not immediately apparent. For the first week of culture small embryos appeared to grow nearly as well on this medium as on Seitz filtered coconut milk. Subsequently a complete absence of root formation in these embryos was observed. The inhibition of root development was the most obvious effect produced by heat sterilization of coconut milk.

Coconut milk contains a factor which is essential to the formation of root and leaf primordia of barley embryos. Lacking this factor, small embryos fail to become further differentiated *in vitro*. The factor is active upon barley embryos only at levels present in nearly undiluted coconut milk.

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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 83, No. 1, pp. 30–34 JANUARY, 1956

A COMPARATIVE STUDY OF THE EFFECT OF CORN (ZEA MAYS) STEEP LIQUOR, BENGAL GRAM (CHICK PEA OR CICER ARIETINUM) STEEP LIQUOR AND RICE (ORYZA SATIVA) STEEP LIQUOR ON THE PRODUCTION OF PENICILLIN ON SURFACE CULTURE

M. F. ISLAM AND K. AHMAD

The discovery that corn steep liquor (c.s.l.), a by-product of starch manufacture from maize, greatly stimulates the production of penicillin (Moyer & Heatley 1941, Moyer & Coghill 1946 a & b) must be regarded as a landmark in the commercial history of penicillin. The factors present in c.s.l. responsible for this effect have never been completely determined. It would appear that a multiplicity of factors are involved; mineral constituents, amino acids, and specific precursors such as phenyl acetic acid (Florey et al. 1949). Many natural products such as vegetable juices, yeast extracts, and molasses, have been tried as substitutes for c.s.l. without complete success (Florey et al. 1949). Other materials tried include cotton seed meal, sardine meal, pea nut oil meal, soybean meal and the like (Perlman 1949).

The present investigation was prompted by an observation of the authors that Bengal gram steep liquor (g.s.l.) prepared according to the method for c.s.l. (Bowden & Peterson 1946) was found more effective in the production of the antibiotic than c.s.l. (Ahmad & Islam 1955). Rice steep liquor (r.s.l.) was also included in this investigation.

Method. *Penicillium notatum* NRRL 1249-B21 and *Penicillium chrysogenum* (Wis) Q176 were used in these experiments. Spores were obtained on test tube slants containing the media suggested by Moyer and Coghill (1946 a & b). The fermentation medium used was also that suggested by these authors, with the difference that c.s.l. was substituted by g.s.l. and r.s.l. and phenyl acetic acid was added in some of the experiments. The mold was grown in surface culture in pyrex glass 500 ml. fermentation flasks using 250 ml. of the medium per flask giving a surface of 11 cm. diameter and having a depth of 3.5 cm. at the centre. The flasks were incubated at $25 \pm 1°$.

Preparation of steep liquor. The method described by Bowden and Peterson (1946) was followed. Suitable amounts of the materials (corn. gram or paddy) were first washed with distilled water and soaked at room temperature (21°) for some time (corn and paddy, 2 days; gram, 1 day). They were then spread over a moist sand bed with occasional spraying with distilled water till the sprouts were nearly half an inch long. This usually

TABLE 1. *Effect of 1% corn, Bengal gram and rice steep liquor solids on the production of penicillin by Penicillium Chrysogenum Q—176 (Wis). The mold was grown in surface culture, the temperature of incubation being 24 ± 1°C. The terms g.s.l. and r.s.l. mean corn, Bengal gram and rice steep liquors respectively.*

(Diameter in mm. of inhibition zone after days of incubation.)

	Days	6	7	8	9	10	11	Difference of diameter of inhibition zones (in mm.) at maximum activity with corn steep liquor as control.
Without phenylacetic acid.	g.s.l.	33	39	42	35	32	–	4
	c.s.l.	24	29	38	30	25	–	–
	r.s.l.	26	30	39	29	23	–	1
With 0.05% phenylacetic acid.	g.s.l.	38	42	48	42.5	38.5	34	4
	c.s.l.	35	39	44	41	37	32	–
	r.s.l.	31	37	43	39	34	29	– 1

took 2–3 days. The sprouted seedlings were then mashed in a mortar and then treated with double its amount of 0.24% sodium sulphite solution. The pH was adjusted to 6. The material was kept at 65–70° for 24 hours and then filtered through muslin. This filtrate on storing in a refrigerator generally left a residue which was decanted. The concentration of the extract was taken to 50% solid by boiling off excess water.

Assay of penicillin produced was done by cylinder plate method originally devised by Heatley (1944) with *B. subtilis* NCIB 3610 as the test organism. Six drops of culture fluid withdrawn from time to time from the culture flasks were added to each of the cylinders. The plates were kept in a refrigerator overnight (10–12 hrs.) before incubation at 37°C. Readings of the diameter of the inhibition zone were taken after 12 hrs. of incubation

TABLE 2. *Effect of 5% corn, Bengal gram and rice steep liquor solids on the production of penicillin by Penicillium chrysogenum Q-176 (Wis). (The mold was grown in surface culture, the temperature of incubation being 25 to 28° C. The terms c.s.l., g.s.l. and r.s.l. mean corn, Bengal gram and rice steep liquors respectively.)*

(Diameter in mm. of inhibition zone after days of incubation.)

	Days	6	7	8	9	10	11	Difference of diameter of inhibition zones (in mm.) at maximum activity with corn steep liquor as control.
Without phenylacetic acid.	g.s.l.	38	42.5	50	44	37	–	7.5
	c.s.l.	29	32.5	42.5	41.5	33.5	26	–
	r.s.l.	32	39	41	48	41.5	37	4.5
With 0.05% phenylacetic acid.	g.s.l.	31	38	42	45	35	–	2
	c.s.l.	28	35	41	43	39	34	–
	r.s.l.	24	29	32.5	35	33	29	– 8

TABLE 3. *Effect of 1% corn, gram and rice steep liquor solids on the production of Penicillin notatum NRRL 1249-B-21. (The mold was grown in surface culture, the temperature of incubation being 24 ± 1°C. The terms c.s.l., g.s.l. and r.s.l. mean corn, Bengal gram and rice steep liquors respectively.)*

(Diameter in mm. of inhibition zone after days of incubation.)

	Days	6	7	8	9	10	11	Difference of diameter of inhibition zones (in mm.) at maximum activity with corn steep liquor as control.
Without phenylacetic acid.	g.s.l.	29	35	42	41.5	34.5	–	3
	c.s.l.	18	26	39	33	29	–	–
	r.s.l.	23	25	36	34	30	–	–3
With 0.05% phenylacetic acid.	g.s.l.	31	38	48.5	42	36	–	3.5
	c.s.l.	25	36	43	39	32	–	
	r.s.l.	27	34	39	33	29	–	–4

and all results reported are means of two runs of experiments done simultaneously under identical conditions.

Results. In table 1 are reported results obtained with *Penicillium chrysogenum* and 1% steep liquor solids with and without phenyl acetic acid. In table 2 are presented the results of similar experiments using 5% steep liquor solids in the media. Tables III and IV show results with *Penicillium notatum* with 1% and 5% steep liquor solids respectively with and without phenyl acetic acid. The experiments were designed only to show the relative potencies of the three steep liquors keping other conditions identical. The maximum production of the antibiotic has been found on the 8th or 9th day of fermentation, in all experiments. From table 1 and 2 it is seen the g.s.l. is superior to both c.s.l. and r.s.l. for production of penicillin by *P. chrysogenum* Q 176 (Wis). This superiority is maintained even on introduction of

TABLE 4. *Effect of 5% corn, gram and rice steep liquor solids on the production of Penicillin notatum NRRL 1249-B-21. (The mold was grown in surface culture the temperature of incubation being 25 to 28°C. The terms c.s.l., g.s.l. and r.s.l. mean corn, Bengal gram and rice steep liquors respectively.)*

(Diameter in mm. of inhibition zone after days of incubation.)

	Days	6	7	8	9	10	11	Difference of diameter of inhibition zones (in mm.) at maximum activity with corn steep liquor as control.
Without phenylacetic acid.	g.s.l.	36	44.5	48	43	38.5	–	7
	c.s.l.	26	32.5	41	33	29	–	–
	r.s.l.	32	39	45	41	35	–	4
With 0.05% phenylacetic acid.	g.s.l.	30	35	39	46	42	38	8
	c.s.l.	26	31	34	38	43	39	–
	r.s.l.	29	33	38	40	36	31.8	2

0.05% phenyl acetic acid in the media as precursor. Rice steep liquor is consistently inferior to both corn and gram steep liquors. Tables 3 and 4 show that when *P. notatum* NRRL 1249-B21 is used the superiority of g.s.l. over the other two is still more pronounced, both in the absence and presence of phenyl acetic acid. It is to be noted that the different steep liquors are made equivalent only in so far as the total solids contained therein are concerned. These steep liquors would, of course, contain soluble amino acids, peptides, aminoacid—amides and dicarboxylic acids besides a host of other organic and inorganic compounds presumably in varying quantities. One might recall that White, Krampitz and Werkman (1945) found that a mixture of histidine arginine, glutamic acid and succinic acid accounted for much of the activity of corn steep liquor. It is to be noted that a difference in one mm. in the diameter of inhibition zone may mean a difference of as much as 30 percent in the production of penicillin (Pharmaceutical Society of Great Britain (1952)).

The reason for the superiority of g.s.l. over others cannot be ascertained unless the detailed analyses of their ingredients are available. It is to be noted that c.s.l. used in these experiments was prepared with corn grown locally and no comparison has been made with corn steep liquor available elsewhere.

It has been noted in the course of these investigations that *P. notatum* NRRL 1249-B21 when grown with c.s.l. gave rise to fluffy white mycelia during the first 6–7 days. The mycelium subsequently turned green on sporulation. This organism, however when grown with g.s.l. or r.s.l. underwent very early sporulation. The yellow colour produced in the media was also deeper in the latter cases.

SUMMARY

1. The stimulating potencies of Bengal gram steep liquor, corn steep liquor and rice steep liquor have been studied in the production of penicillin by *P. chrysogenum* Q 176 (Wis), and *P. notatum* NRRL 1249–B-21 using Moyer and Coghill's lactose medium with and without phenyl acetic acid.

2. It has been found that Bengal gram steep liquor is superior to corn and rice steep liquors.

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STUDIES ON THE GENETICS OF ASCOBOLUS STERCORARIUS (BULL.) SCHRÖT.[1]

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The species used in this investigation, *Ascobolus stercorarius* (Bull.) Schröt., is a member of the Pezizales, an order of operculate discomycetes. The fungus is heterothallic with compatibility controlled by a single pair of allelomorphs. Each mature ascus contains eight dark-brown, uninucleate ascospores which may be uniseriate, biseriate, or irregularly disposed (figs. 1 and 2). The ascospore wall is at first hyaline, becoming purple and finally dark-brown and at the same time thickened and striated. This development is accompanied by an increase in ascospore size. Oidia, which are also produced, may function either as asexual spores or as fertilizing agents in sexual reproduction.

Few genetic studies have been attempted in the genus *Ascobolus* primarily because of the difficulties in germinating spores and obtaining fruiting bodies in culture. Recently, however, Yu (1954) was able to overcome both obstacles. She found that the ascospores of several species, including those of *A. stercorarius,* respond to treatment with dilute alkali followed by incubation at 37° C. By varying her method somewhat, still higher percentages of ascospore germination were obtained, thus making the present experiments feasible.

The genetic data reported in this paper were obtained by the analysis of various crosses involving the following three loci: (1) mating-type (*A, a*), (2) spore abortion (l^+, l), (3) tan spore color (t^+, t).

The three pairs of factors behave like true mendelian allelomorphs in that any ascus heterozygous for a pair of these factors will produce four spores of each genotype. The mutant alleles at the latter two loci will be referred to as ascospore mutants, since their effect is manifested in the ascospore stage (figs. 7–31). Thus any spore carrying the l factor will remain infantile; that is, it will be colorless and smaller than a normal mature spore. In addition such spores never germinate, and after a period of several weeks disintegrate in the ascus. The tan-spored factor apparently acts at a later stage in ascospore maturation since t spores, although tan in color are normal in size and germinate readily. The fact that gene action is autonomous, taking place in the ascospore stage and having an immediate

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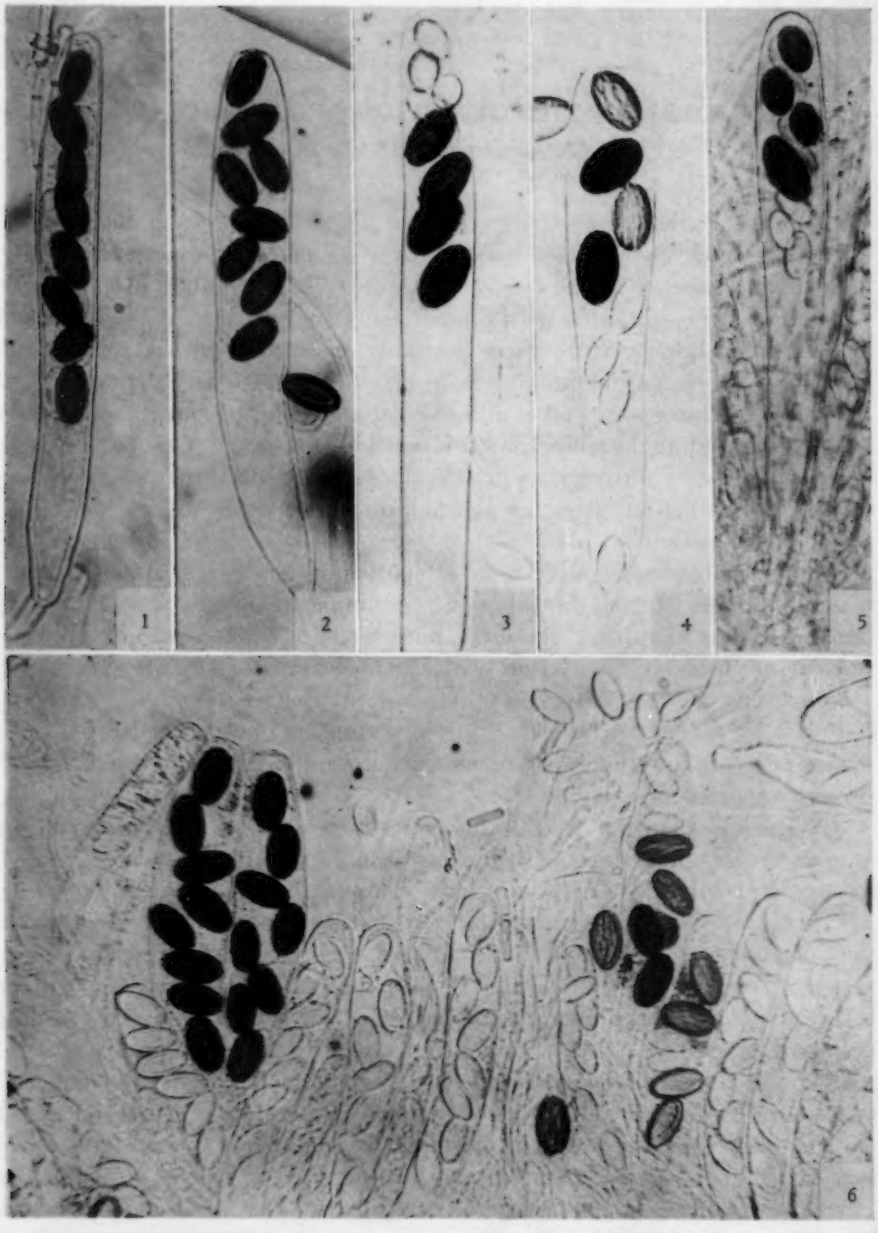
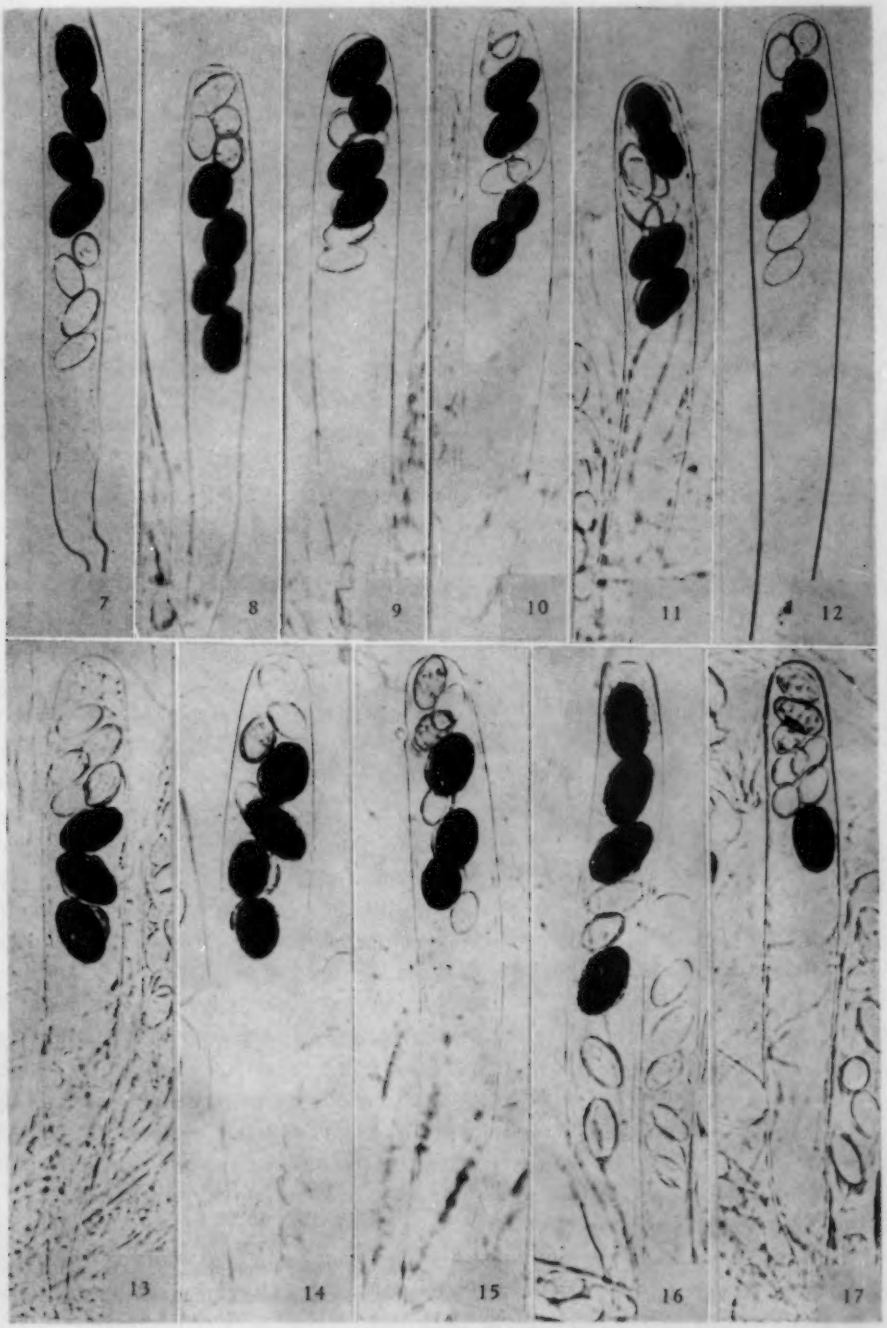
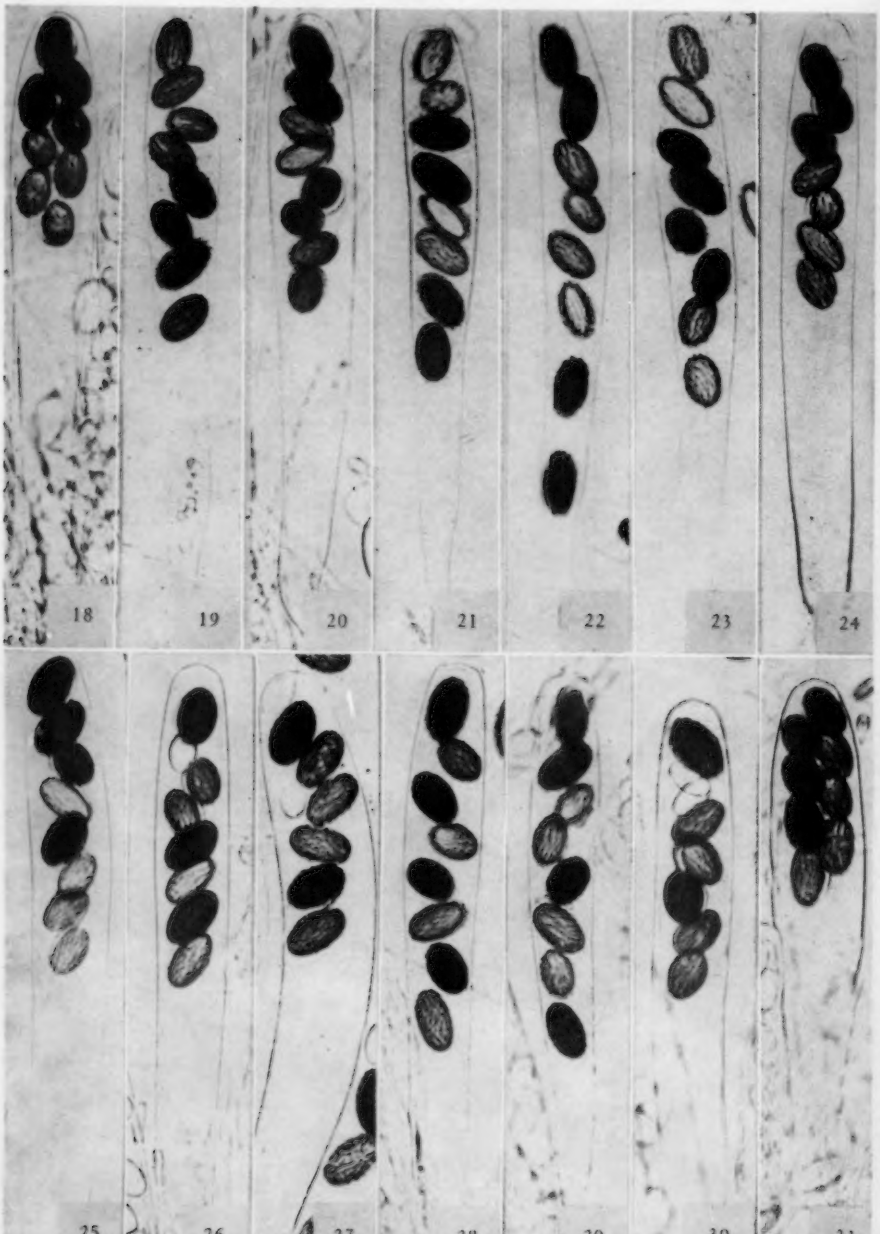


FIG. 1.—Wild-type ascus with uniseriate spores. ×500. FIG. 2.—Wild-type ascus with partially biseriate spores. ×500. FIG. 3.—Class I tetratype ascus from the cross L(*alt*$^+$) × T-1 (*Al*$^+$*t*). ×600. FIG. 4.—Class II (for *t* locus) tetratype ascus from the cross L (*alt*$^+$) × T-1 (*Al*$^+$*t*). ×600. FIG. 5.—Ascus with seven spores, one a giant spore. ×500. FIG. 6.—Asci from a single apothecium of the cross (*at*$^+$ *l*$^+$) + (*atl*) × (*At*$^+$ *l*$^+$). ×500.



FIGS. 7–17. Asci from the cross L(*al*) × W-1 (*Al*$^+$). × 600. FIGS. 7–13.—Class I asci. FIGS. 14–15.—Class II asci. FIG. 16.—Class III indeterminate ascus (MI or MII-As). FIG. 17.—Class IV ascus.



FIGS. 18–31. Asci from the cross T-2 (*at*) × W-1 (*At*$^{+}$). × 600. FIGS. 18–24.—Class I asci. FIGS. 25–26.—Class II asci. FIGS. 27–28.—Class III determinate asci (MII-As). FIG. 29.—Class III indeterminate ascus (MII-As or MII-Sy). FIG. 30.—Class III indeterminate ascus (MI or MII-As). FIG. 31.—Class IV ascus.

phenotypic effect, obviates the necessity for ascus dissection and greatly facilitates the genetic analysis of such asci.

Before a valid interpretation of the tetrad analysis data can be made in any organism there must be a precise knowledge of the cytological events accompanying the maturation divisions. In these eight-spored ascomycetes two of the primary events which can affect the data are (1) the relationship of the second-division spindles, *i. e.*, whether they overlap or are superposed and (2) the relationship of the third-division spindles and the occurrence of post-mitotic nuclear migrations or ascospore shifting.

In *Neurospora crassa* and *N. sitiphila* cytological studies indicate that there is no overlapping of spindles, nuclear migration or ascospore displacement. The ascospore pattern with respect to any pair of allelomorphs is an orderly, exact replica of the meiotic segregation pattern. Thus a monohybrid cross yields six ascus patterns: type MI, segregation in the first meiotic division (figs. 7 and 8); type MII-As, asymmetrical second-division segregation (figs. 9 and 10); type MII-Sy, symmetrical second-division segregation (figs. 11 and 12). Since homologous centromeres separate in the first meiotic division (Ryan 1943) the uncorrected map distance for the gene-centromere interval is one-half the proportion of second-division segregation asci (Lindegren 1933).

In *Ascobolus stercorarius* the situation is more complex. The nuclear divisions in the ascus have been studied by several cytologists (Dangeard 1907; Fraser and Brooks 1909; Björling 1941). Their illustrations show no overlapping of the two second-division or of the four third-division spindles, although the latter may be closely clustered and the possibility of overlapping does exist. The two second-division spindles may be parallel to the long axis of the ascus or slightly oblique but never transverse. The third-division spindles may assume almost any position. If third-division spindle overlapping did occur its immediate consequence would be the appearance of more than six patterns in a sample of asci. Post-mitotic nuclear migration or a shifting of ascospores could give the same result. One or a combination of these three alternatives must occur in this species, because in addition to the six expected patterns one finds a large number (40–60%) and variety of anomalous ascus patterns (figs. 14–16, 25–31). The data to be presented in this paper will show that the six expected patterns represent a biased ascus sample. Consequently, all ascus patterns must be included in the calculation of a statistic such as the perentage of post-reduction.

The purposes of this paper are: (1) to present a method for determining gene-centromere distances in fungi with anomalous ascus patterns, (2) to describe some effects of low temperature on the nuclear divisions in the ascus of *Ascobolus stercorarius*, and (3) to make a study of linkage relationships.

In addition there will be a discussion of the theoretical upper limit of

post-reduction, 66.7%, and of several papers which report a frequency at or above this limit.

Materials and methods. The procedure used in ascospore germination and the methods of culture were, in general, similar to those described by Yu (1954).

The medium used for culturing the fungus vegetatively was composed of 0.4% yeast extract, 1.0% glucose, and 2.5% agar; whereas apothecia were obtained on a medium composed of 0.3% yeast extract, 2.5% agar, and filter paper (one 9 cm. disk per plate).

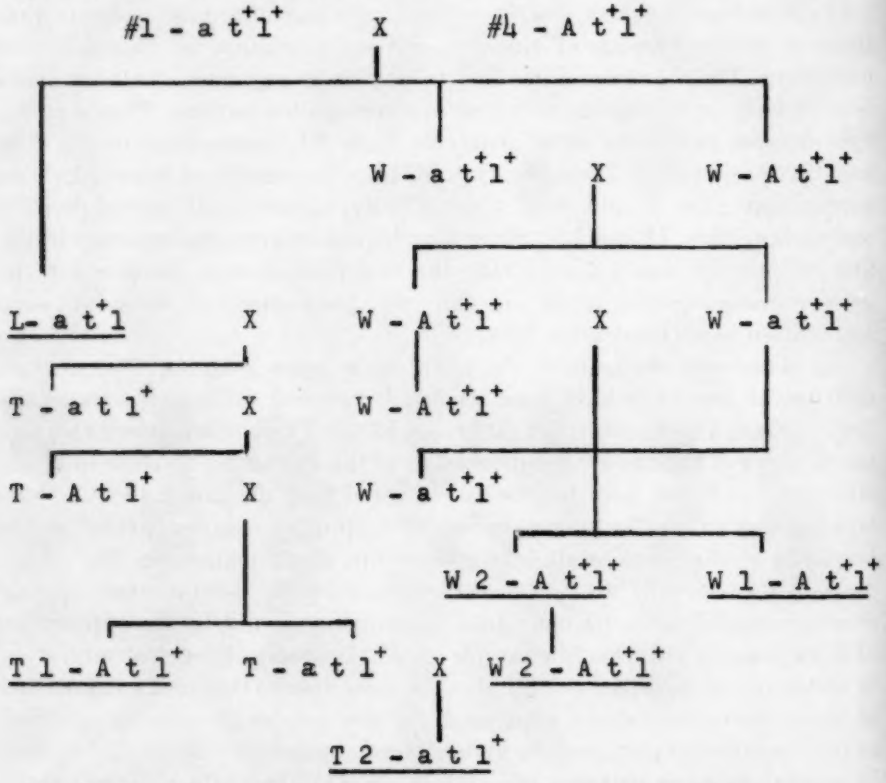


FIG. 32. The lineage and genotypes of the various isolates used in this study.

All single ascospore cultures referred to in this report were derived from Dr. Yu's original No. 1 and No. 4 strains which were isolated from apothecia found on cow dung obtained from the New York Zoological Park. The lineage and genotypes of the various isolates are outlined in figure 32.

The five single-ascospore cultures underlined in the pedigree table were used in obtaining the data reported in this paper. Both ascospore mutants occurred spontaneously. The original L strain (at^+ l) was derived from a

single germinating ascospore in a cluster of eight spores obtained from a cross between strains 1 and 4. These spores differed from the wild-type in that they were non-striated and somewhat larger. Since all subsequent ascospores carrying the *l* factor failed to germinate it was not possible to obtain further isolates carrying this factor. The first T strain was isolated as a single-ascospore culture from the cross L $(at^+l) \times W(At^+ l^+)$. All subsequent T strains were obtained from various T × W crosses.

As was pointed out in the introduction, ascus dissection and germination of the ascospores were necessary only in experiments requiring mating-type analysis. Consequently two general methods of ascus analysis were used.

The first method involves ascus dissection. The two compatible strains were inoculated at opposite sides of a Petri dish containing the yeast extract-cellulose medium and incubated at room temperature (20–28° C). After 7–12 days single, ripe apothecia were taken from the culture and transferred to a drop of distilled water on a microscope slide. The apothecium was crushed by a cover slip, the latter was then removed, and the apothecial remnants were washed off the slide onto a plate of agar. Individual asci were then transferred to plates of 4 or 5% plain agar and stored for 10 days at room temperature. This period allowed for the completion of ascospore maturation. On the tenth day the asci were transferred to fresh 4 or 5% agar plates (three asci per plate), the spores dissected out in order, and the plates kept at room temperature for three days. The dishes were then flooded with freshly prepared 0.17% NaOH solution for 20 minutes and placed in an incubator at 37° C. until the spores had germinated. The 10-day maturation period was required because immediate treatment with NaOH in some way injures the younger spores and results in a very low percentage of germination. The three-day period following dissection is to allow the spores to settle on the agar surface and thus lessen the possibility of their being washed off. A few germinated spores will be found each day over a period of two weeks after treatment with NaOH.

The second method of ascus analysis involves the direct observation of segregation in the ascus (Bistis and Olive 1954). The three strains used were W1, T2 and L. The W1 strain was inoculated in the center of plates of yeast extract-cellulose agar, three days after which the colony reached the edge of the dish. The next step was to scrape off masses of aerial growth—consisting mostly of oidia—from the surface of a six-day old culture of the T2 or L strain and to spread these oidia over the surface of the W1 strain. During the subsequent 24 hour period (at room temperature) the early stages of sexual reproduction occurred and many incipient apothecia could be seen at the end of this period. The plates were then placed in three temperature control rooms set at 24–25° C., 14–16° C., and

6–7° C. respectively. The advantage of the foregoing procedure is that a large number of fruiting bodies of about the same age can be obtained simultaneously and be subjected to the various temperatures just prior to ascus development. At 24–25° C. mature asci were obtained six days after seeding. At 14–16° C. the time of maturation was ten days and at 6–7° C., about twenty-three days.

After these three incubation periods single apothecia were placed in a drop of 10% glucose solution on a microscope slide and the asci were spread out under a cover slip. The sugar solution was used rather than water because in the latter the asci will absorb water, build up a high turgor pressure and burst. In making the counts all of the mature asci in any one packet of asci were scored in order to insure against any personal bias.

Results. Determination of the percentages of second-division segregation for the loci *l* and *t* and the compatibility locus. The *l* locus. The following data were obtained by scoring asci from the cross L × W1 incubated at 24–25° C. and at 14–16° C. In order to make possible a clear, concise presentation of the data the myriad types of asci found were grouped into these four major classes:

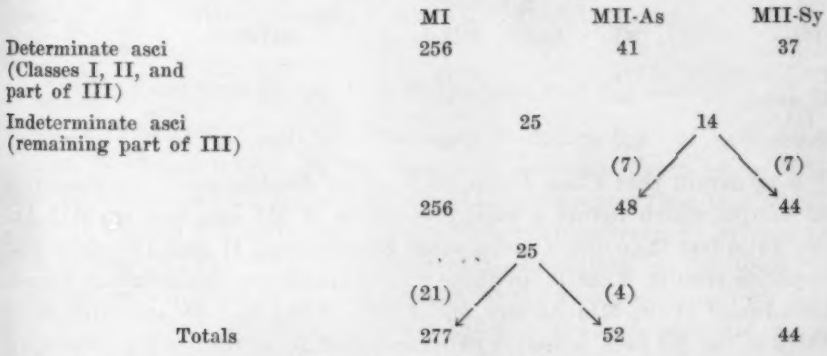
(a) Spore pattern determinate
Class I—no overt spore displacement
Class II—one overt primary displacement
(b) Spore pattern determinate or indeterminate
Class III—more than one primary, one or more secondary, one tertiary displacement, or more than one of the wild-type spores aborted
(c) No interpretation possible
Class IV

The diversity of ascus types is the product of two major factors. First, genotypically wild-type (l^+) spores may sometimes abort and become phenocopies of the mutant spores (l). Whenever two or more of the wild-type spores are aborted the ascus is automatically placed in class III or IV. Second, the ascospores are often displaced. A primary displacement involves the exchange of positions of two adjacent spores; in a secondary displacement the two sister spores of one spindle are interposed between the two spores of a second spindle; in a tertiary displacement the two sister spores of one spindle are separated by the two spores of a second spindle and by one of the spores of a third. Such displacements can be visualized with the aid of the theoretical models indicated in figure 33.

Although the discussion and diagrams are based on spindle overlapping, identical results could be obtained by post-mitotic nuclear migrations or ascospore shifting.

Returning to the four classes outlined above, it is evident that in Classes I and II the ascus segregation type is determinate; that is, each ascus can be classified as MI, MII-As, or MII-Sy (figs. 7–15). In class III some asci are determinate, others indeterminate (fig. 16). Figure 16 could be interpreted as an MI, but it could just as easily be classified as an MII-As segregation type. Class IV includes a variety of rare ascus types (fig. 17). It should be noted that each of the four classes includes asci of each of the three basic segregation types, and the subsequent presentation of the data will show a correlation between the MI : MII-As : MII-Sy ratio and the particular class.

From the above discussion it should be evident that Classes I, II and the determinates of Class III offer no special problem since each ascus clearly belongs to one of the basic segregation types. Class IV can be ignored in the calculations because of its rarity (0.5% at 24–25° C. and 1.9% at 14–16° C.). The indeterminate asci of Class III are the critical group in the calculation of the percentage of second-division segregation and other proportions such as the MII-As : MII-Sy ratio. The correct interpretation of this group is the crux of the problem of gene-centromere analysis in this fungus. The problem is to determine the ratio in which these indeterminate Class III asci should be apportioned to each of the two possible segregation patterns. The criterion chosen in determining this ratio is the relative proportions of MI, MII-As, and MII-Sy asci found in Classes I, II, and the determinates of III. The following diagram outlines the procedure followed:



The three segregation types in the determinate asci are in the ratio 6.92 : 1.11 : 1.00 and the percentage of second-division segregation is 23.4%. However, there are two groups of indeterminate asci, the MI or MII-As (25 asci) and the MII-As or MII-Sy group (14 asci). The procedure is to apportion the latter group into the two segregation classes in the ratio 1.11 : 1.00 (7 : 7). Thus the overall ratio is now 256 : 48 : 44 (5.82 : 1.09 : 1.00). The ratio 5.82 : 1.09 is used as the best estimate of the MI : MII-As proportion in the population and the 25 indeterminate asci of this group are apportioned accord-

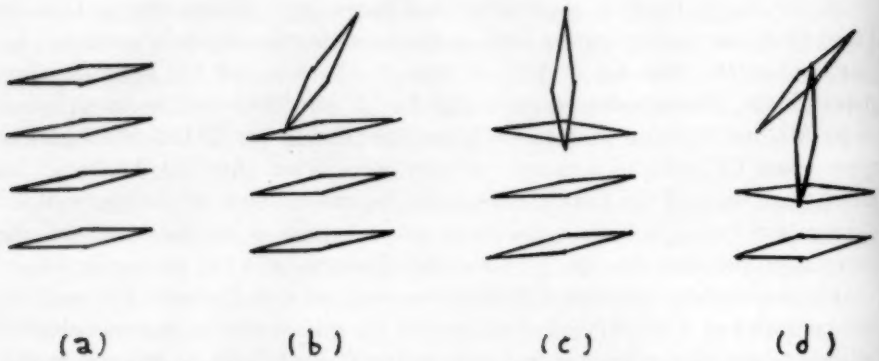


FIG. 33. Graphic representation of four representative third-division spindle orientation patterns: a) No ascospore displacement. b) a primary spore displacement. c) a secondary displacement. d) a tertiary displacement.

ingly (21:4). Consequently, the totals now are 277, 52, 44, while the ratio is 6.30:1.18:1.00 and the percentage of second-division segregation is 25.7%. In summary, the two effects of including these indeterminate ascus patterns in the calculations are an increase in the value for second-division segregation from 23.4% to 25.7% and a change in the MII-As:MII-Sy ratio from 1.11:1.00 to 1.18:1.00.

These two effects are much more evident if the data are presented in the following way:

Class	MI	MII-As	MII-Sy	% post-reduction
I	179	12	21	15.7%
II and III	98	40	23	39.1%
Totals	277	52	44	25.7%

It is apparent that Class I (no overt spore displacement) represents a biased sample which favors a high proportion of MI asci and an MII-As:MII-Sy ratio less than one. On the other hand classes II and III yield just the opposite results. That is, in these two overt spore displacement classes are found most of the MII-As asci, about 50% of the MII-Sy asci, and about one-third of the MI asci. A simple explanation of these results is represented in figure 34.

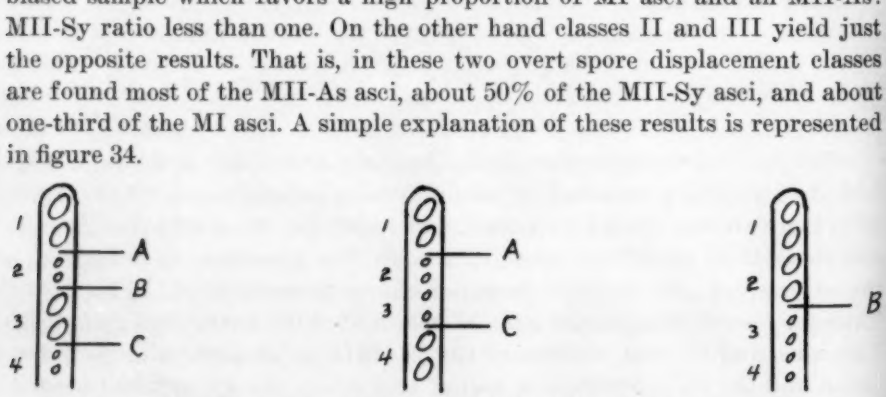


FIG. 34. A, B, and C represent the points where third-division spindle overlapping will result in an overt spore displacement. The four spindles are numbered 1, 2, 3, and 4.

From figure 34 it is clear that MII-As asci will have an overt displacement if any two of the four third-division spindles overlap; MII-Sy asci will have an overt displacement if spindles 1 and 2 or 3 and 4 overlap; MI asci will have an overt displacement only if spindles 2 and 3 overlap. This situation could well be the explanation of the data obtained. But again, post-mitotic nuclear migration or ascospore shifting could give the same results.

Frequency of post-reduction at 24–25° C. With the above discussion in mind one may now attempt to interpret the data. The data in table 1 support the view that the effect of third-division spindle overlapping is detected most often in MII-As, less frequently in MII-Sy, and least often in MI asci. Consequently in the overt displacement classes (II and III) the frequency of MII-As is significantly greater than that of MII-Sy asci

TABLE 1. *Summary of the counts from seven samples of approximately 375 asci each from the cross L (al) × W1 (Al⁺) at 24–25° C.*

Class*	Total No. of Asci	Type MI	Type MII			% MII Types		
			MII-As	MII-Sy	Total	MII-As	MII-Sy	MII
I	1655	1329	124	202	326	7.5	12.2	19.7
II	734	492	147	95	242	20.0	13.0	33.0
III	345	143	117	85	202	33.9	24.7	58.6
Totals	2734	1964	388	382	770			
%		71.8	14.2	14.0	28.2			

* Class IV asci numbered only 13 in the total sample of 2747 asci.

($\chi^2_1 = 12.42$ $P < .01$) and the frequency (uncorrected) of post-reduction is high (0.33 and 0.586). In the class with no overt displacement, Class I, just the reverse is true, the number of MII-Sy is greater than that of MII-As asci ($\chi^2_1 = 18.66$ $P < .01$) and the frequency (uncorrected) of post-reduction is low (0.197). The combining of these classes in the totals yields the expected equality in the frequencies of the two post-reduction segregation types (388 to 382) and also gives the most accurate estimate of second-division segregation, 28.2%. The reason for calculating separately the percentage of MII-As and MII-Sy asci will be made clear in the discussion of the data from the experiment at 14–16° C.

Simplicity of presentation was the reason for giving the data in terms of MI, MII-As, and MII-Sy rather than listing all six expected patterns (figs. 7–12). The frequencies of the six patterns have been conserved in the original data, and it can be stated that there is no evidence of biased segregation.

The original data were analyzed statistically to see if the percentages of second-division segregation in the seven samples were in agreement with one

another. The heterogeneity χ^2 test for the segregation division gave a value of 4.77 with six degrees of freedom; thus $0.50 < P < 0.70$, indicating that the data are homogeneous in this respect.

Of the 10,988 wild type spores observed (4×2747) only 52 had aborted (0.5%).

Frequency of post-reduction at 14–16° C. Reference to table 2 shows that, as at 24–25° C, Classes II and III give a significantly greater frequency of MII-As than MII-Sy asci ($\chi^2_1 = 44.6$ $P < .01$) a condition that tends to be counterbalanced by the ratio found in class I ($\chi^2_1 = 6.12$ $0.01 < P < 0.02$). The percentages of second-division segregation for the three classes at 14–16° C. show the same relationship to each other as at 24–25° C.

Despite this superficial resemblance of the data obtained at the two different temperatures the totals reveal some major differences. At 24–25° C.

TABLE 2. *Summary of the counts from five samples of approximately 375 asci each from the cross* $L(al) \times W1(Al^+)$ *at 14–16° C.*

Class*	Total No. of Asci	Type MI	Type MII			% MII Types		
			MII-As	MII-Sy	Total	MII-As	MII-Sy	MII
I	906	670	99	137	236	11.0	15.1	26.1
II	539	301	135	103	238	25.1	19.1	44.2
III	359	100	188	71	259	52.4	19.8	72.2
Totals	1804	1071	422	311	733			
%		59.4	23.4	17.2	40.6			

* Class IV asci numbered only 37 in the total sample of 1841 asci.

the MII-As:MII-Sy ratio is 1:1 (14.2:14.0%) and the percentage of second-division segregation is 28.2%. At 14–16° C. the MII-As:MII-Sy ratio is 1.35:1.00 (23.4:17.2%) and the percentage of post-reduction is 40.6%. The 12.4% increase in post-reduction appears to be due to two distinct phenomena—an increase (from 14.0 to 17.2%) in the proportion of MII-Sy asci ($\chi^2_1 = 8.90$ $P < .01$) and an even greater increase (from 14.2 to 23.4%) in the MII-As asci. Consequently the MII-As to MII-Sy ratio is no longer 1:1 ($\chi^2_1 = 16.82$ $P < .01$). The immediate problem was to determine whether any single hypothesis could explain both phenomena. Several hypotheses based on results in other fungi are considered.

Whitehouse (1942), in discussing the possibility of second-division spindle overlapping in *Neurospora*, observed that such overlapping will result in an interchange between MI and MII-As asci. If post-reduction is any value less than 66.7% the MII-As class is smaller than MI; therefore, this interchange will result in an increase in the former and a decrease in the

latter. Type MII-Sy asci, on the other hand, will be unaffected by the overlapping of second-division spindles. This hypothesis could explain the observed MII-As:MII-Sy ratio (1.35:1.00) as well as part of the 12.4% increase in post-reduction. But since MII-Sy asci are unaffected by this overlapping, the increase in the frequency of this class (14.0 to 17.2%) would not be accounted for.

Whitehouse and Haldane (1946) in discussing the deviation of the observed MII-As:MII-Sy ratio from the expected ratio in *Neurospora sitophila* and in *Bombardia lunata* (Zickler's data) suggested the possibility that non-random orientation of the two pairs of centromeres at the second meiotic division, with respect to the long axis of the ascus, might account for

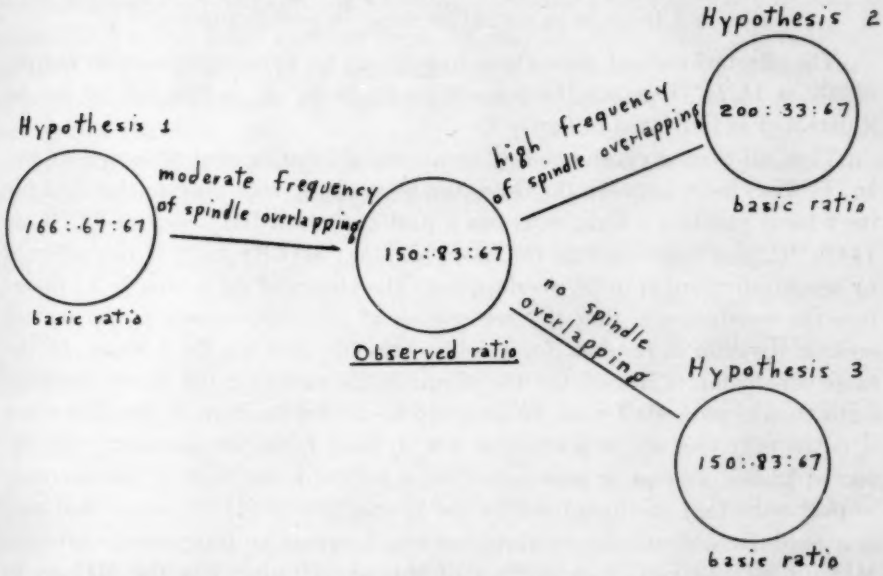


FIG. 35. Diagram showing the effect of the three suggested hypotheses on a theoretical ascus sample of 300 at 14–16° C. when the proportion at 24–25° C. is 200:50:50.

the excess of MII-As over MII-Sy segregations. But again the increase in the frequency of MII-Sy asci in the present data would not be accounted for in this manner.

The third alternative is that there is an actual increase in post-reduction (due to a temperature effect on the frequency and/or distribution of chiasmata) at 14–16° C. In *Podospora anserina,* Rizet and Engelmann (1949) found such an increase for several loci. But this hypothesis would not explain the discrepancy between the two second-division segregation types in our data.

Thus, used alone, none of these hypotheses will explain our data. Con-

sequently, one is compelled to combine these hypotheses and to determine which combination best explains the results:

The three composite hypotheses are:

(1) second-division spindle overlapping occurs, 1:1 is the basic MII-As:MII-Sy ratio, and there is an actual increase in post-reduction.

(2) second-division spindle overlapping occurs, the basic MII-As:MII-Sy ratio is not 1:1 there being a preponderance of MII-Sy, there is no actual increase in post-reduction.

(3) no second-division spindle overlapping occurs, the basic MII-As:MII-Sy ratio is not 1:1, there being a preponderance of MII-As asci, and there is an actual increase in post-reduction.

The effect of each of these three hypotheses on a theoretical ascus sample of 300 at 14–16° C. when the proportion at 24–25° C. is 200:50:50 can be illustrated as indicated in figure 35.

Thus all three hypotheses lead to a ratio similar to that observed at 14–16° C. The choice between the three can be made by referring to the data for the *t* locus (table 4). This locus has a post-reduction frequency of 66.7% at 14–16° C. and consequently the basic MII-As:MII-Sy ratio is not affected by second-division spindle overlapping. The observed ratio was 1:1; therefore the conclusion is that the orientation of the centromeres at the second meiotic division is random for the chromosome bearing the *t* locus. If the same conclusion is drawn for the chromosome carrying the spore abortion locus then hypothesis 1 must be accepted as the explanation of the data since it is the only one which postulates a 1:1 basic ratio. In summary, the observed 12.4% increase in post-reduction is believed due to an actual increase in post-reduction (as measured by the proportion of MII-Sy asci) and also to second-division spindle overlapping which causes an interchange between MI and MII-As asci. A side effect of this overlapping was the MII-As to MII-Sy ratio of 1.35:1.00.

The original data were analyzed statistically to determine whether the MII-Sy frequencies of the five samples were in agreement with one another. The five samples are homogeneous ($\chi^2_4 = 5.12$, Yates' correction $0.20 < P < 0.30$). However, when sample 3 was tested (12.9%) against the population value (17.2%) the difference was just significant at the 5% level. The data give no evidence of biased segregation. Of the 7,364 wild-type spores observed (4×1841) 229 had aborted (3.1%).

An attempt was made to score asci from a culture grown at 6–7° C., but the abortion of wild-type spores was so prevalent that it was impossible to obtain an adequate sample. Thus another effect of lower temperature is to increase the frequency of abortion of wild-type spores.

The *t* locus. The data presented here were obtained by scoring asci from the cross T-2 (*at*) × W1 (*At*$^+$) grown at 24–25°, 14–16°, and 6–7° C.

As in the case of the spore-abortion locus the asci in this cross could be grouped into the four previously outlined classes. Examples of these various classes are given in figures 18–31. The diversity of ascus types is due to the two major factors, the abortion of *l*$^+$ spores and ascospore displacement.

Again in Classes I and II the ascus segregation pattern is determinate and offers no special problem. In Class III both determinate (figs. 27 and 28) and indeterminate (figs. 29 and 30) patterns are found and hence Class III is the critical group in the mapping of this gene-centromere interval. However, in a T × W cross the apportioning of the indeterminates into the two possible segregation types is much simpler. Since the frequency of post-reduction for this locus appears to be 66.7%, the three basic segregation patterns, MI, MII-As, MII-Sy, have the same frequency (0.33); hence the indeterminate asci can be apportioned equally to each of the two possible segregation patterns.

Frequency of post-reduction at 24–25° C. With the above discussion in mind the data of table 3 can now be interpreted.

TABLE 3. *Summary of the counts from eight samples of approximately 375 asci each from the cross T-2 (at) × W1 (At*$^+$*) at 24–25° C.*

Class*	Total No. of Asci	Type MI	Type MII			% MII Types		
			MII-As	MII-Sy	Total	MII-As	MII-Sy	MII
I	1588	654	359	575	934	22.6	36.2	58.8
II	857	223	363	271	634	42.4	31.6	74.0
III	528	65.5	278.0	184.5	462.5	52.7	34.9	87.6
Totals	2973	942.5	1000.0	1030.5	2030.5			
%		31.7	33.6	34.7	68.3			

* Class IV asci numbered only 17 in the total sample of 2990 asci.

Since the observed percentage of second-division segregation is 68.3% the three segregation patterns would be expected to be in a 1:1:1 ratio. Nevertheless in Class I the frequencies of these patterns are in the order, MI > MII-Sy > MII-As. However, as with the 24–25° C. data for the spore-abortion locus, the relation of these frequencies in Classes II and III, MII-As > MII-Sy > MI, counterbalances the Class I data. Thus in the totals the three patterns are in a 1:1:1 ratio (942.5:1000.0:1030.5). Again the statement that an overt spore displacement occurs most often in MII-As, less frequently in MII-Sy, and least often in MI asci is supported by the data.

The original data were tested statistically in several ways. The eight samples were found to constitute a homogeneous population with respect to percentage of post-reduction ($\chi^2_7 = 7.11$ $0.30 < P < 0.50$). The observed aggregate percentage of post-reduction, 68.3%, does not differ significantly from the theoretical limit of 66.7% ($\chi^2_1 = 3.56$ $0.05 < P < 0.10$), and accordingly each of the three basic patterns can have the frequency, 0.33. However, in one sample (#6) the percentage of second-division segregation (73.2%) was significantly above 2/3 ($\chi^2_1 = 8.40$ $P < .01$).

As a whole, the original data gave no evidence of biased segregation. However, there was a discrepancy in the frequencies of the two MII-As patterns with respect to the positions of the wild-type and mutant spore pairs in the ascus. The frequencies of the two kinds of spore arrangements were 542 (fig. 20) and 451 (fig. 21), which differed significantly from the expected 1:1 ratio ($\chi^2_1 = 8.4$ $P < .01$).

Of the 23,920 spores observed (8×2990), 171 had aborted (0.7%). There was no significant difference in the frequency of abortion for the two spore genotypes, t^+ and t.

Frequency of post-reduction at 14–16° C. The data at this temperature (table 4) again show the reciprocal relationship of Class I and Class II, Class III asci.

TABLE 4. *Summary of the counts from two samples of approximately 375 asci each from the cross T-2 (at) × W1 (At⁺) at 14–16° C.*

Class*	Total No. of Asci	Type MI	Type MII			% MII Types		
			MII-As	MII-Sy	Total	MII-As	MII-Sy	MII
I	384	160	101	123	224	26.3	32.0	58.3
II	200	64	78	58	136	39.0	29.0	68.0
III	175	42.0	81.5	51.5	133.0	46.6	29.4	76.0
Totals	759	266	260.5	232.5	493			
%		35.0	34.3	30.7	65.0			

* Class IV asci numbered only 10 in the total sample of 769 asci.

The two samples were tested statistically to determine if they are in agreement with one another with respect to post-reduction frequency. The heterogeneity $\chi^2_1 = .92$ $0.30 < P < 0.50$ indicates that the data are homogeneous in this respect. The difference in the observed MII-As and MII-Sy frequencies (260.5 to 232.5) is not significant ($\chi^2_1 = 1.60$ $0.20 < P < 0.30$). The observed post-reduction, 65.0% does not differ significantly from the theoretical limit, 66.7% ($\chi^2_1 = 1.00$ $0.30 < P < 0.50$), and thus each basic pattern again has a frequency of 0.33.

Although the data of table 4 appear to be quite different from that of table 2, both are compatible with hypothesis 1 (proposed to explain the data of table 2). Because of the equality of the MI and MII-As segregation patterns for the *t* locus, second-division spindle overlapping would not tend to increase the proportion of MII-As asci, and consequently no increase in the percentage of post-reduction due to this overlapping would occur. The second postulate mechanism; *i.e.*, an actual increase in post-reduction due to an effect on the frequency and/or distribution of chiasmata, apparently has no effect on the post-reduction frequency of the *t* locus. The question of whether this fact is related to the upper limit of post-reduction will be discussed later.

The original data gave no evidence of biased segregation. Of the 6,152 spores observed (8×769), 336 had aborted (5.5%).

Frequency of post-reduction at 6–7° C. The data obtained at 6–7° C. (table 5) again show Class I and Class II, Class III interrelationship.

TABLE 5. *Summary of the counts from two samples of approximately 250 asci each from the cross T-2 (at) × W1 (At⁺) at 6–7° C.*

Class*	Total No. of Asci	Type MI	Type MII			% MII Types		
			MII-As	MII-Sy	Total	MII-As	MII-Sy	MII
I	129	52	29	48	77	22.5	37.2	59.7
II	111	41	26	44	70	23.4	39.6	63.0
III	280	67.5	119.0	93.5	212.5	42.5	33.4	75.9
Totals	520	160.5	174.0	185.5	359.5			
%		30.9	33.4	35.7	69.1			

* Class IV asci numbered only 18 in the total sample of 538 asci.

In the statistical analysis the two samples gave a value of $\chi^2_1 = .07$ $0.70 < P < 0.80$ for post-reduction indicating that the data are homogeneous. The observed post-reduction frequency (69.1%) does not differ significantly from the theoretical 66.7% limit ($\chi^2_1 = 1.42$ $0.20 < P < 0.30$). Obviously the MII-As : MII-Sy ratio is the expected 1 : 1 ratio.

Although the frequency of post-reduction for the *t* locus is the same for all three temperatures there are some differences among the three tables. The Class I : Class II ratios for the three temperatures 24–25°, 14–16°, and 6–7° C. are 1.83 : 1, 1.92 : 1 and 1.11 : 1, respectively. Apparently at 6–7° C. the probability of an overt spore displacement is greater than at the two higher temperatures.

The original data gave no evidence for biased segregation.

Of the 4,304 spores observed at 6–7° C. (8×538), 865 had aborted. Thus

the percentages of spore abortion at 24–25°, 14–16°, and 6–7° C. are 0.7%, 5.5% and 20.1% respectively.

The compatibility locus (*A*,*a*) at 20–28° C. The gene-centromere interval for this locus was measured by the analysis of asci from the cross L(*al*) × W2(*Al*$^+$) by means of ascus dissection. Since the *l* gene was involved in this cross, at most only four of the eight spores in each ascus germinated (the *l* spores aborting). Consequently only those asci showing first-division segregation for the *l* locus (figs. 7 and 8) were used in determining the post-reduction frequency of the compatibility locus. In other words, if in an ascus showing post-reduction for the *l* locus the four *l*$^+$ spores proved to be 2*A* and 2*a* in mating-type it would be impossible to determine whether this represented pre- or post-reduction for the compatibility locus.

In determining the *A*, *a* locus post-reduction frequency, only those asci in which at least three of the wild-type spores had germinated were included in the scores. Asci with less than three germinated spores were grouped under separate columns.

TABLE 6. *Analysis of asci from the cross L(al) × W-2(Al*$^+$*) at 20–28° C.*

	Asci with 3–4 spores germinated					Asci with less than three spores germinated			
Meiotic div. in which A, a segr.	MI		MII			Not determinable			
Spore genotypes	4a	4A	2a:2A	Totals	1 or 2a	1 or 2A	1a:1A	No spore germ.	Totals
No. of asci	56	33	13	102	19	10	1	25	55

No sterility was encountered: every single-ascospore culture fruited with one of the tester strains.

Of 157 asci tested, three or more of the wild-type spores germinated in 102 asci. Since these spores were always from the same half-ascus the frequency of post-reduction is merely the proportion of asci yielding both mating-types (13/102 = 12.8%). This post-reduction percentage for the compatibility locus should be considered as tentative because of the large number (55) of unanalyzable asci.

A tendency to be noted is the preponderance of asci with recombinant (*al*$^+$) spores. This unexpected result will be discussed in connection with table 7.

Tests of linkage among the three loci. The *l* and *t* loci. Obviously, the most direct method for determining linkage would be to make the cross L (*alt*$^+$) × T-1(*Al*$^+$*t*) and test whether the ratio of non-parental ditype to parental ditype differs from a 1:1 ratio. However there is no way of distinguishing *t* spores from light-brown or immature *t*$^+$ spores in different

asci. Thus an ascus with four light-brown spores could be either a recombinant or parental ditype.

Consequently the asci had to be considered as unordered tetrads and the data related to the function:

$$f(III) = x + y - \frac{3xy}{2}$$ (Perkins 1949; Whitehouse 1949)

where x and y represent the frequencies of second-division segregation of the two loci concerned and f(III) is the frequency of tetratype tetrads. The 24–25° C Class I post-reduction frequencies for the loci (*t* and *l*) give:

$$f(III) = 19.2 + 58.9 - \frac{3(19.2)(58.9)}{2}$$

$$f(III) = 61.1\%$$

Thus in a dihybrid cross the expectation is that 61.1% of the Class I asci should be tetratypes (fig. 3). Of 297 Class I asci scored (at 24–25° C.) there were 181 tetratype and 116 ditype asci. This gives an observed percentage of tetratype tetrads of 60.9%, which is nearly identical with the expected value.

A confirmation was obtained by using the total frequencies of post-reduction at 24–25° C. for each locus, calculating the expected frequency of tetratype asci, and then scoring all asci from a dihybrid cross. Thus:

$$f(III) = 28.2 + 68.3 - \frac{3(28.2)(68.3)}{2}$$

$$f(III) = 67.6\%$$

Of 322 asci, 216 were tetratype and 106 were ditype. The tetratype percentage $\frac{216}{322}$ is 67.1% and is compatible with the expected value of 67.6%.

The conclusion from both samples is that the two loci, *l* and *t*, segregate independently.

The *l* and compatibility loci. The data presented in table 7 represent that of table 6 in addition to a number of asci in which the *l* locus had segregated in the second division. Samples 7 and 8 were obtained by the previously outlined ascus dissection method, the others by various modifications of this method.

Since the *l* locus was involved in this cross each ascus contained only four spores capable of germinating. Thus in sample 1 the 68 spores were taken from 17 asci, the 40 of sample 2 from 10 asci, etc. In the statistical analysis if only one of the four wild-type spores of an ascus had germinated it was given a value of one, if two had germinated each spore was given a value of one-half, for three each was given a value of one-third, and for four the value was one-fourth.

From this same cross 267 ascospores were taken at random. The results were: 83 parental, 87 recombinant, 97 unanalyzed.

Intuitively the data appear to represent three distinct populations. Samples 1 to 6 show a high recombination percentage and a large proportion of unanalyzed spores; samples 7 and 8 have approximately 50% recombination and few unanalyzed spores; and the random ascospore samples have a 50% recombination and many unanalyzed spores.

When tested statistically the data gave the following results. With respect to recombination percentage, samples 1 to 6 constitute a homogeneous population ($\chi^2_5 = 1.24$ $0.90 < P < 0.95$) as do the data for samples 7 and 8 ($\chi^2_1 = 1.07$ $0.30 < P < 0.95$). However the heterogeneity χ^2_1, for the two aggregate samples (1 to 6 and 7,8) was 4.31 (Yates' correction, $0.02 < P < 0.05$). Furthermore each of the three aggregate samples was tested against the expected recombination percentage (50%). Samples 1 to 6 have $\chi^2_1 = 10.08$ $P < .01$, samples 7,8 gave $\chi^2_1 = 0.1$ $0.70 < P < 0.80$, while for the random ascospore sample $\chi^2_1 = 0.1$ $0.70 < P < 0.80$.

TABLE 7. *Spore frequencies from the cross* $L(al) \times W\text{-}2(Al^+)$

Sample	Total No. of Spores	Spore Genotype			% Recombinant Spores
		Not Determined	Parental (Al^+)	Recombinant (al^+)	
1	68	36	12	20	62.5
2	40	15	6	19	76.0
3	40	15	3	22	88.0
4	84	42	17	25	59.5
5	40	15	9	16	64.0
6	96	60	9	27	75.0
7	192	27	85	80	48.5
8	192	28	72	92	56.1
Total	752	238	213	301	
%			41.4	58.6	

At the present time no final conclusion has been reached regarding these results.

A problem that arose during this investigation concerned the heterogeneity of the fusion nuclei; *i.e.*, whether all of the fusion nuclei in a single fruiting body are derived from a single pair of nuclei and are therefore necessarily of the same genotype. The use of ascospore mutants in crosses simplifies the problem considerably. From the cross L $(at^+l) \times$ Tl(Atl^+) a seven-spored ascus was isolated. One of the spores was larger (apparently binucleate) and when crossed it fruited only with the A tester (At^+l^+). In some of the resulting apothecia two types of asci were found (fig. 6). Apparently the giant spore was heterokaryotic for each of the spore color

factors, the genetic formulae of the two nuclei being at^+l^+ and *atl*. In the ascogonium following plasmogamy and in the ascogenous hyphae there would be two kinds of paired nuclei, $At^+l^+ + at^+l^+$ and $At^+l^+ + atl$. Consequently, the two types of asci found in figure 6 show that the fusion nuclei in the asci of a single fruiting body can originate from more than one nuclear pair.

Discussion. The various phases of the nuclear cycle in *Ascobolus stercorarius* have been studied by numerous cytologists, the only previous genetic studies being the limited analyses of mating-type segregation by Dowding (1931) and Green (1931). This unilateral approach has resulted in conflicting reports on various aspects of the cycle.

A recent paper by Olive (1954) offers a possible explanation for certain of the contradictory reports. In the genus *Ascobolus* the two heterothallic species, *A. stercorarius* and *A. furfuraceus*, are superficially very similar in appearance but intersterile. Because of their similarity and the consequent failure of previous investigators to discriminate between the two it is frequently impossible to determine which species was being studied. Thus if the nuclear cycles of the two species differ, this failure of discrimination could lead to contrary reports. Only in the descriptions of Green and Dowding is it fairly clear that the species studied was *A. stercorarius*.

A brief review of some of these earlier papers combined with our observations may help to clarify the situation further. Ames (1930) reported that single-ascospore cultures of *A. stercorarius* produced a few apothecia but that when two such cultures were crossed at random there was in 50% of the cases a great increase in the number of fruiting bodies formed. Hence he described the species as being hetero-homothallic. Green (1931) also reported what she termed "good" or "mature" apothecia in single-spore cultures. Björling (1941) observed that apothecia from monospore cultures of *A. stercorarius* did not differ from those formed in crosses. On the other hand, Dowding (1931) maintained that this species is strictly heterothallic and that fruiting bodies are formed only in crosses between two compatible strains. She also showed that mites functioned in the dissemination of oidia and that these mites could carry oidia from one strain to a complementary one growing in another Petri dish. These displaced oidia functioned somehow in sexual reproduction and as a result the culture fruited. Dowding concluded that the reported fruiting of single-ascospore cultures was due to oidial contamination caused by mites and flies. Our studies have fully confirmed the ability of oidia to function in sexual reproduction leading to the development of fertile apothecia.

Two other possible explanations must also be considered in connection with the appearance of apothecia in monospore cultures. Dangeard (1907) in an extensive investigation of many fungi studied the life cycle of *A. furfuraceus*, including the cytology of the ascus. One of his figures is a seven-

spored ascus in which one of the spores was larger and binucleate. We too have found such asci (fig. 5) and have confirmed the binucleate condition of the giant spores both cytologically and genetically. Ten of these spores have been germinated but none proved to be heterokaryotic for mating-type; at least none was self-fertile and when mated with the two tester strains fruited with only one. The second alternative is based solely on one of our observations. Of the several hundred single-ascospore strains studied, the vast majority produced some type of hyphal aggregations. In some instances they resembled true apothecia. If these apothecium-like bodies were examined early enough a coiled ascogonium could be found, and later a layer of paraphyses embedded in a golden yellow mucilaginous layer developed. The only apparent morphological difference between these bodies and true apothecia was the absence of asci in the former. Hence, the so-called apothecia found in monospore cultures by earlier investigators may well have been such sterile bodies. *A. stercorarius* must consequently be considered as strictly heterothallic.

One apparent exception has been observed. A single-ascospore culture did produce many fertile apothecia containing eight-spored asci. But on the first subculture these apothecia were limited to the area adjacent to the inoculum, the peripheral portion of the colony remaining sterile. Portions of the original (self-fertile) culture were crossed with the two tester strains but fruiting occurred only with the *A* tester. Finally, several asci were isolated from the apothecia found in the original self-fertile culture. Five of the spores from one of these asci germinated and developed into self-sterile cultures. When tested three proved to be of the *a* mating-type and two, *A*. The most probable explanation is that the original ascospore was binucleate and heterokaryotic for mating-type, but very quickly the *A* nuclei were eliminated. Nevertheless this observation is not incompatible with one of the basic characteristics of heterothallism; *i.e.*, that the fusion nucleus in the ascus must always be heterozygous for the compatibility factors.

The second point of controversy concerns the ploidy of the ascus fusion nucleus. Welsford (1907) and Fraser and Brooks (1909) claimed that nuclear fusion occured both in the ascogonium and in the young ascus in *A. furfuraceus*, resulting in a tetraploid ascus fusion nucleus. The latter authors also stated that the third nuclear division in the ascus was brachymeiotic, a division considered necessary for a return to the haploid condition in the nuclei of the ascospores. On the basis of our genetic data it can be stated that the fusion nucleus of *A. stercorarius* is diploid. One of the more direct proofs of this diploidy is as follows. If the nucleus were tetraploid, then for all loci the proportion of asci in which all eight spores alternated as to genotype (fig. 28) would be the same as or greater than that of MI asci. The data for the three loci studied show no such relationship in the two

proportions. The first two divisions in the ascus are meiotic, the third is mitotic.

The third question involves the migration of nuclei through the cells of the ascogonial coil to the primary ascogonial cell. Dangeard (1907) claimed that no such nuclear migration occurs, whereas several of the others thought that it does occur. Our observations on the mechanics of fertilization verify the migration of nuclei from oidium to trichogyne and into the main body of the ascogonium. These observations will be presented in a subsequent paper.

Ascomycete chromosome maps have been drawn basically from two types of data. Gene-gene intervals have been determined by the frequencies of ascospore genotypes, whereas gene-centromere intervals have been mapped from the frequencies of post-reduction ascus patterns. The principal requirement for the accurate mapping of gene-centromere intervals is that the observed frequency of second-division segregation (as determined by scoring the various ascus patterns) must be identical with the actual frequency of post-reduction (as determined by the number and relationship of the chiasmata in the interval). In addition more apparent sampling errors must be averted. The conclusions concerning the effect of temperature on post-reduction must also be related to the above precautions. The data for the spore-abortion factor at 14–16° C. (as compared with 24–25° C.) indicate: (1) increased abortion of wild-type spores, (2) second-division spindle overlapping which is responsible for part of the increase in the observed post-reduction frequency, (3) an actual increase in post-reduction (due to some alteration in the number and/or relationship of the chiasmata in this gene-centromere interval) which is responsible for the remainder of the increase in the observed percentage of second division segregation.

The data for the tan-spored factor indicate that as the temperature decreases from 24–25° to 14–16° to 6–7° C. (1) there is an increase in the abortion frequency of both tan and wild-type spores, (2) there is no increase in the observed or actual frequency of post-reduction, which is 66.7% at all three temperatures, and (3) at 6–7° C. there is a much higher proportion of asci with an overt spore displacement than at the more moderate temperatures (24–25° and 14–16° C.).

The most significant result is that for the spore-abortion locus there is an actual increase in post-reduction frequency at the lower temperature (14–16° C.), whereas for the tan-spored locus the post-reduction frequency is the same at all three temperatures. The three most probable explanations for such an increase are (1) an increase in the mean number of chiasmata in the *l*-centromere interval, (2) an increase in chiasma interference in the interval, or (3) some type of chromatid interference leading to a preponderance of three-strand double crossovers. But if the effect of low temperature were the same for both intervals (*l*-centromere and *t*-centromere), on the

basis of alternatives (2) and (3) one would expect an increase in the post-reduction frequency of the *t* locus rather than its remaining constant at 66.7%. Based on the assumption that the effects of low temperature on the two intervals are basically the same, alternative (1) is accepted as the most probable explanation of the results. That is, at the lower temperatures there is an actual increase in the mean number of chiasmata in both intervals which results in an increase in the post-reduction frequency of the *l* locus and a static frequency for the *t* locus.

It is interesting to note that a marked increase in crossing-over within the 10–15° C. temperature range has been reported in other organisms. Plough (1917) found a marked increase in crossing-over at 13° C. and 31° C. between certain genes located in the second chromosome of *Drosophila melanogaster*. White (1934) in a cytological study of three species of locusts reported a high frequency of chiasmata at diplotene and diakinesis at 10–15° C.

There have been two reports of the relationship between temperature and post-reduction in fungi. Rizet and Engelmann (1949) studied several loci in the four-spored species *Podospora anserina* and found that at low temperatures (10–11° C.) the post-reduction frequency for some loci decreased, whereas for others it increased. Hüttig (1931) reported that the post-reduction frequency of the compatibility factors in the bipolar smut *Ustilago Hordei* varied directly with the temperature (up to a certain point).

Before a further discussion of these papers, it should be noted that a mathematical expression for the upper limit (66.7%) of second-division segregation was first given by Mather (1935). In the absence of positive chiasma interference and chromatid interference the limit cannot be depassed. In a recent review article Barratt, Newmeyer, Perkins and Garnjobst (1954) have cited several reported instances of post reduction (or tetratype) frequencies greater than 66.7%. A brief re-examination of the data of these papers is considered appropriate here.

Two of the papers cited in the review article of Barratt, Newmeyer, Perkins and Garnjobst (1954) listed tetratype frequencies above 66.7% in two yeasts (Lindegren 1949, Leupold 1950). In both instances, however, a χ^2 test reveals that the data are entirely compatible with the theoretical upper limit of post-reduction.

However, the data in the other two papers (if they have been interpreted correctly by the authors) do show a frequency significantly above 66.7%. Rizet and Engelmann (1949) studied the four-spored *Podospora anserina* and reported post-reduction frequencies in excess of 66.7% for several loci. On the basis of their results (more than 90% second-division segregation for two unlinked loci) they concluded that a very high degree of chiasma interference must occur in several of the chromosomes. The greatest complication

in the genetic study of this four-spored species is that each spore is binucleate and that the genetic analysis frequently involves the determination of nuclear genotypes in heterocaryons. Just which ascospore patterns represent post-reduction and which represent pre-reduction depends on the orientation of the spindles in the three nuclear divisions in the ascus. At the present time there is no reliable description of the ascus cytology of *P. anserina*.

The second report of excessive post-reduction is that of Hüttig (1931) in *Ustilago Hordei* in connection with the mating-type locus. However, in Hüttig's method for mating-type determination there is the possibility of a selection in favor of those tetrads which he interpreted as representing post-reduction. In addition, his method did not always discriminate between MI and MII-As segregations.

There have been several reports of eight-spored Ascomycetes in which loci have been found to show post-reduction frequencies compatible with the 66.7% limit. In all cases it is certain that the observed percentage of second-division segregation (as determined by scoring the various ascus patterns) corresponds almost exactly to the actual frequency of post-reduction (as determined by the number and relationship of the chiasmata at prophase I). Mathieson (1952) studied *Chromocrea spinulosa* cytologically and genetically. For two loci she found post-reduction frequencies compatible with the ⅔ limit; 65.1% (of 2161 asci) and 62.3% (of 512 asci). Olive (personal communication) has found a percentage of approximately 67.0 for a spore color factor in the homothallic species *Sordaria fimicola*. Zickler (1934) in *Bombardia lunata* found 64.0% second-division segregation (for 8204 asci). However he did not include all observed asci in the scoring and in addition reported that a small percentage of asci did show spore displacement. In the light of our experience with the tan-spored factor, it is probable that if Zickler had attempted to classify all of the asci the percentage would have gone up to 66.7%. Finally, in *Neurospora crassa*, many loci have been found (on several of the chromosomes) which have a percentage of post-reduction compatible with ⅔. However, the sample is rather small for a number of these loci.

In summary, in the only two species where the reported values for post-reduction have clearly depassed 66.7%, it is significant that the validity of the interpretation of the data is an open question; whereas in a number of species where there is little doubt as to the interpretation of the data, a number of loci have been found which segregate at but not above this limit.

SUMMARY

1. *Ascobolus stercorarius* is heterothallic with compatibility controlled by a single pair of alleles (*A*,*a*).

2. The following phases of the nuclear cycle have been ascertained: the fusion nucleus is diploid, and there is no brachymeiosis; in a single apothecium the fusion nuclei of the asci may be of different genotypes; following plasmogamy there is nuclear migration from oidium to trichogyne and into the main cells of the ascogonium.

3. The frequency of post-reduction for three loci has been determined at several temperatures:

(a) spore-abortion factor (*l*)
24–25° C.—28.2%
14–16° C.—40.6% (uncorrected for second division spindle overlapping)

(b) tan-spore factor
24–25° C.—68.3%
14–16° C.—65.0%
6–7° C.—69.1%

(c) mating type (*A*,*a*)
20–28° C.—12.8% (approximate)

4. There is no conclusive evidence of linkage between any of the loci studied.

5. Other low temperature effects (at 14–16° C. and 6–7° C.) were (1) greater incidence of spore abortion and overt spore displacement, and (2) increased overlapping of second-division spindles.

6. A procedure for analyzing gene-centromere data obtained from a fungus in which anomalous ascus patterns occur is presented.

7. The upper limit (66.7%) of post-reduction is discussed in relation to various reports of loci depassing this limit.

Acknowledgements. The writer wishes to express his appreciation to Professor L. S. Olive under whose direction this study was made and to Professor F. J. Ryan for his interest and suggestions in many aspects of the work.

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A COMPARATIVE STUDY OF HAWAIIAN SPECIES OF THE FERN GENUS DOODIA

ROBERT F. BLASDELL

The representatives of the fern genus *Doodia* R. Br. in Hawaii have been a source of some concern to taxonomists since the recognition of *Doodia lyoni* Degener in 1934. In part, this may be traced to Degener's Latin diagnosis which recognizes a species status for *D. lyoni* entirely on the basis of its small size as compared with the larger *D. kunthiana* Gaudichaud. Another source of concern involves several statements in Degener's English description which do not always hold true, probably due to the lack of really representative collections of the plant.

The author has therefore felt it necessary to bring together all information bearing on the subject to date in order to clarify conflicting reports. Furthermore, an anatomical study of the leaves and sori of both entities has been undertaken in order to make a discussion of the validity of *D. lyoni* as complete as possible.

Degener's description and comments on *Doodia lyoni* leave much to be desired but are ameliorated to a certain extent by his excellent illustrations. Besides the objectionable distinguishing of a species primarily on size, there are in addition several inconsistencies in the English description which are pointed out by Skottsberg in his analysis of the situation (1942). In the first place Degener gives a maximum of 18 cm. for the length of the frond. Skottsberg, however, cites examples ranging from 24 to 30 cm. in length, while I have examined two specimens with frond lengths of 20 and 23.5 cm. Secondly, Degener states that there is only one row of areolae on each side of the pinna. Skottsberg, on the other hand, points out the presence of a second row in some specimens. Lastly, Degener characterizes the position of the sorus as being near to, and hiding, the costa. This may be true in a majority of cases but Skottsberg points out that "one and the same specimen will occasionally bear single fronds with slightly wider segments and smaller sori more distant from the midrib." Other characters which have been used by these two authors to distinguish *Doodia lyoni* from *D. kunthiana* have been the shorter stipes, fewer pinnae, and the fewer but larger sori of *D. lyoni*.

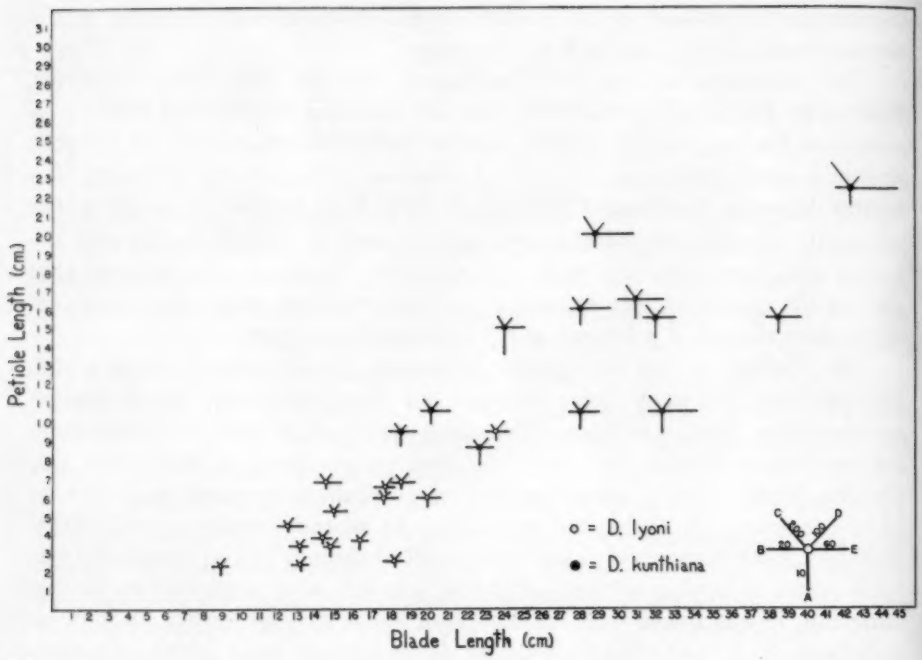
This study has been made possible through the generosity of Dr. Warren H. Wagner, Jr. who has placed at my disposal an extensive collection of the two species of *Doodia* which was made by him during two trips to Hawaii in the summers of 1947 and 1949. He has also supplied pertinent information

concerning the ecology of the species and has given aid and criticism in the arrangement of the paper and the drawings.

The investigation has been conducted strictly with dried material. Specimens available in the University of Michigan Herbarium have been examined for supplementary data used in the species description of *D. lyoni* and in a small quantitative study of selected characters in the form of a scatter diagram. Anatomical investigation has been limited primarily to the pinna, its venation, its cellular composition, and the sori. Consequently the tannic acid-ferric chloride stain (Foster 1934) has been utilized profitably during the course of this investigation. The drawings have been made entirely with the aid of a Bausch and Lomb microprojector.

The ecology of the two species differs in certain respects. The plants available for this study were collected on three islands of the Hawaiian group: Oahu, Maui, and Kauai. They were obtained without exception from regions located in the "C" and "D" vegetation zones of Ripperton and Hosaka (1942) which receive an annual rainfall of from 40 inches to 60 inches or more. The collections were made at altitudes ranging from 1000–3700 feet. Both ferns grow in soil with a pH of around 7.0. Although the two ferns occur in similar habitats, *D. lyoni* is much more restricted in its distribution. It was found without exception along streams, either on vertical rock faces or on rocky banks or slopes. *D. kunthiana,* while inhabiting these same localities, is in addition found in a much wider range of habitats. Open rocky woods, shaded rocks in woods, and rocky banks along roads and trails all appear to supply the necessary conditions for its existence. *D. lyoni* is the rarer of the two species with an apparently narrower ecological tolerance, while *D. kunthiana* is common and widespread in the Hawaiian Islands.

As has already been pointed out, an important character used by Degener to contrast the two entities was the relative size. The difference in size holds true to a great extent in mature plants as is demonstrated in the accompanying scatter diagram (fig. 1). From each of the collections, the two largest plants were selected, and the largest frond of each was chosen for the quantitative study. This diagram shows a distinct segregation on the basis of size with the exception of one *D. lyoni* plant which is within the range of *D. kunthiana.* Even this abnormally large specimen, however, is fairly close to the upper range of the other *D. lyoni* samples. Also the number of stalked pinna pairs seems rather significant: *D. lyoni,* on the one hand, is limited to 1–2 pairs of stalked pinnae, while *D. kunthiana* ranges from 3–7 pairs (only one frond had just two pairs). The relation of stipe length to blade length is fairly consistent although there is an occasional overlap: the ratio of stipe to blade in the case of *D. kunthiana* ranged from 1.0:1.4–1.0:3.1 with an average ratio of 1.0:2.07. On the other hand, *D. lyoni* maintained a ratio varying from 1.0:2.1–1.0:5.5 with an average ratio of 1.0:3.48.



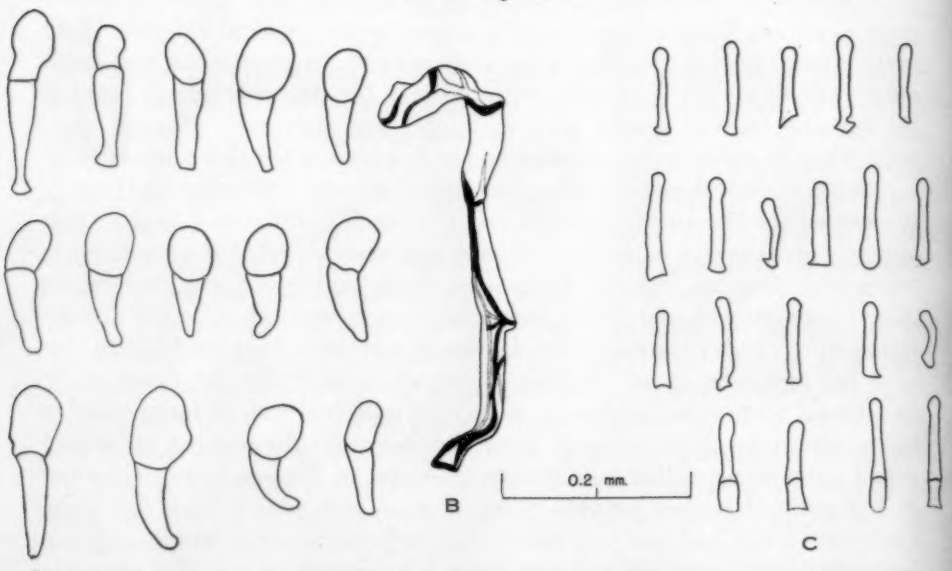


FIG. 1. Scatter diagram showing distribution and range of seven characters in *Doodia kunthiana* and *D. lyoni:* A. Number of stalked pinna pairs. B. Width of median segment (mm.). C. Width of blade (mm.). D. Number of segment pairs of any kind. E. Length of median segment (mm.) FIG. 2. Hair types present on lower laminar surface of *Doodia kunthiana* and *D. lyoni:* A. Capitate hair with globose terminal cell and elongate basal cell. B. Structure transitional between capitate hair and typical scale. C. Linear hairs of 1–2 cells.

Thus there is indicated a definite tendency in *D. lyoni* toward a relatively short stipe. The length of segments is, as a rule, greater in *D. kunthiana* than in *D. lyoni* as is also the width of the segments. However, there is some overlap in these last two characters.

Even though these gross characters seem to indicate the presence of two specifically distinct taxa, perhaps the most rewarding and convincing evidence obtained in the course of this study has arisen through an anatomical investigation of the pinnae, specifically through the study of the hair types, venation pattern, and indusial structure.

The presence of hairs on these two ferns has not received much attention from earlier workers. Degener does not even mention their existence in his discussion of *D. lyoni*. The first mention of this condition is found in Skottsberg's analysis where he quotes a translated note by Christensen referring to *D. lyoni,* as follows, "and from *D. caudata* as well as from *D. media* it further differs by the densely glandulose indusia, the lower side (of the frond) also being glandulose." Skottsberg's own comments on *D. lyoni* account for the remainder of our information on these hairs: "The glands on the lower surface are stipitate and will be found especially along the base of the indusium and on the costa and rhachis. Their density varies."

Two main types of hairs are present on the pinnae of the species concerned. The first of these and probably the most noticeable of the two is the *capitate hair* (fig. 2, A). It is composed typically of an oval or globose terminal cell, and a basal cell which varies greatly in the extent of its elongation. Also, the terminal cell frequently stains more deeply than the basal cell which thus may indicate in the globular cell a glandular function. The distribution of this type of hair on the pinna is extensive, but its frequency varies, depending on the particular specimen. This hair occurs most frequently on the costa, indusium, and on the veins, although it may often also be observed on the lamina between the veins, being evidently on all parts of the lower surface. The marginal area is the only region of the lower surface of the pinna which may ever lack this type of hair. A probable intermediate between this type and the typical scale is sometimes present in the form of a hair comprising three or more cells (fig. 2, B) which is most prevalent on the midrib but has been observed also on the indusium and lamina.

The second type of hair existing on the lower surfaces of the pinnae is the *linear hair* (fig. 2, C). It occurs as a 1–2-celled hair, the cells being greatly elongated. Distribution of this type is more limited than that of the capitate hair. The greatest profusion of this type is reached on the indusia of *D. lyoni* where there may be 50 or more hairs on one indusium. It is found less frequently on the midrib, along the veins, and in the marginal area. Here an important point of distinction between the two species has come to light. Upon examination of the entire collection of *Doodia* it has been found

that hairs are completely absent from the marginal area of the pinnae in the case of *D. kunthiana*, while *D. lyoni* consistently bears hairs on its pinna margins to a greater or lesser degree. These marginal hairs are plainly visible under a dissecting microscope, especially on the greener (probably younger) fertile fronds in the case of dried material. Their frequency, however, varies from abundance to isolated and distantly scattered hairs. Linear hairs have not been observed on the lamina between the veins in either species.

It has been evident from the beginning of this study that the relative development of the two hair types may be used to distinguish the species. Therefore, an attempt has been made to show statistically the hair ratios in the two species. One fertile pinna was examined from each of 12 locality collections (6 of *D. kunthiana* and 6 of *D. lyoni*).[1] Five microscopic fields (at 100× magnification) were chosen at random for each pinna and the hairs were counted. Since the linear hairs occur predominantly on the indusium and the capitate hairs are present both on the indusium and on the lamina, a standardizing procedure was needed. Accordingly, each field was divided equally between an area of indusium and an area of lamina. The frequency ratio of hairs in the case of *D. kunthiana* ranged from 9.2–1.2 (capitate) : 0.8–0.0 (linear) with an average ratio of 4.5 : 0.3 for the six collections. Thus the capitate hair is predominant in *D. kunthiana*. On the other hand, the hair frequency ratio of *D. lyoni* ranged from 0.2–2.8 (capitate) : 32.0–8.6 (linear) with an average ratio of 0.9 : 22.0, for the six collections. In the latter case, therefore, the linear hairs are predominant, and this is a direct reversal of the condition in *D. kunthiana*. In *D. lyoni* the nature of the pubescence of the ventral surface of the pinna is carried over to the rachis which is normally hairy also. On the other hand, the rachis of *D. kunthiana* is essentially glabrous, at most having only scattered hairs of the peculiar many-celled type mentioned above and illustrated in figure 2, B.

The venation pattern of the pinnae does not exhibit any striking basis

[1] *Collections examined* (all collected by W. H. Wagner, Jr. and deposited in the University of California Herbarium): *Doodia kunthiana*—5024, open rocky Koa woods with *Nephrolepis*, 1500 ft. alt., ½ mile E. of Pohakea Pass, Waianae Mts., Oahu; 5116, on rocky banks, Kawaiiki Ditch Trail, alt. 1000–1080 ft., Kawailoa, Koolau Range, Oahu; 5172, shaded rocks in woods, Waihou Spring Reserve, alt. 3700 ft., East Maui; 5197, rocky woods, Piihalo Gulch, alt. 1900 ft., East Maui; 5455, dryish rocky hillsides, kukui woods, upper part of first main gulch ("Kaawa Gulch"), alt. 1000–1250 ft., E. of Kaupakuhali, Oahu; 5481, rocky woods, Kawaikoi stream, first ford along road, alt. 3300 ft., Napalikona Forest Preserve, Kauai. *Doodia lyoni*—5033, shaded vertical rock along stream 3.5 miles from Waimano House, alt. 1200 ft., Koolau Mts., Oahu; 5117, shaded vertical rocky banks beside streams, alt. 1000–1080 ft., Kawaiiki Ditch Trail, Kawailoa, Koolau Range, Oahu; 5196, damp shaded vertical rock faces along stream, Piihalo Gulch, alt. 1900 ft., East Maui; 5484, overhanging rocky bank along Kawaiwoi stream near first ford along road, alt. 3300 ft., Napalikona Forest Preserve, Kauai; 5728, vertical rocky banks along forks of Wahiawa just above Alexander Dam, Kauai; 5828, vertical rock bank above Tulumahu Stream in the water reserve below the summit of Konahuanui (Honolulu side), Koolau Mts., Oahu.

for distinguishing the two species although there are tendencies which often aid in their identification. Both have a row of areolae on each side of the midrib. In both ferns also, the veins terminate just short of the sinuses of the dentate margin. Many of the larger *D. kunthiana* fronds possess very irregularly toothed pinna margins and an increase in branching of the vein system results, adjusting for the increase in number of sinuses (fig. 4, E_1–E_4). Because of the crowding of these veins on the lamina it is perhaps logical to expect that some of the veins will anastomose and form a second

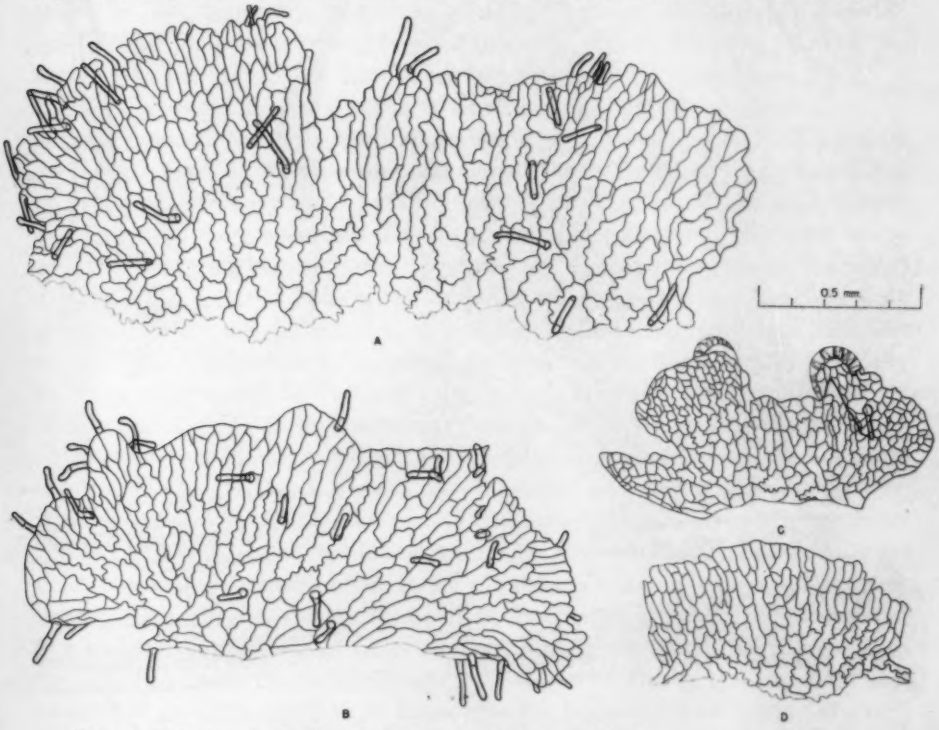


FIG. 3. Indusia: A, B. *Doodia lyoni;* C, D. *D. kunthiana.*

rank of areolae. The condition of two ranks of areolae is very frequent in *D. kunthiana,* usually forming a definite second row when present (fig. 4, E_2), and is utilized in most descriptions of that species. *D. lyoni,* however, also may possess this characteristic, but to a much lesser extent. When present, this second rank of areolae in *D. lyoni* does not form a definite row, but occurs as isolated and widely scattered aerolae.

Correlated with the venation pattern is the position of the sorus. The fertile vein bearing the sorus is formed by the anastomosing of two adjacent veins. Thus every anastomosing pair of veins may potentially form a fertile vein, although this does not always occur. An areole on a fertile pinna is

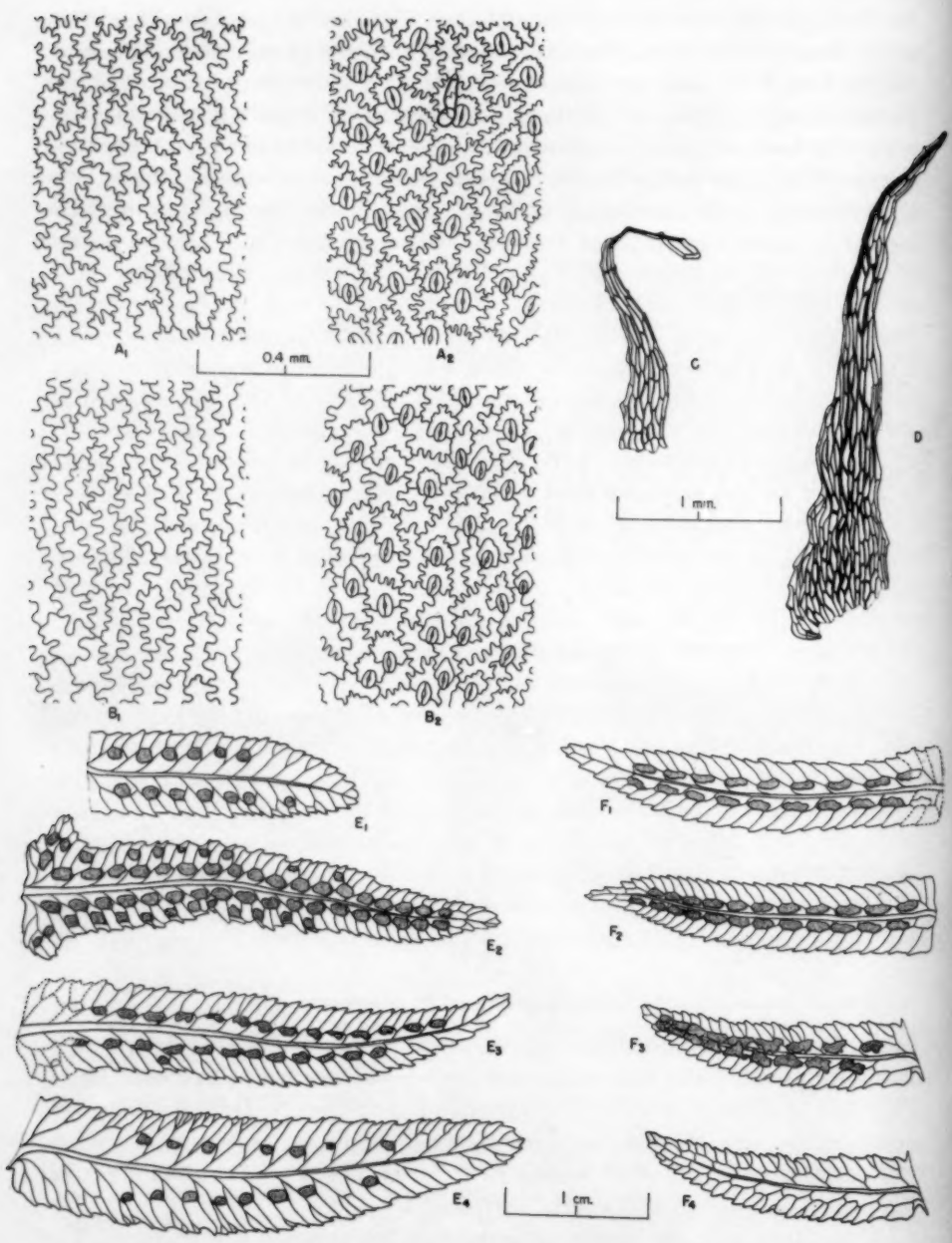


FIG. 4. A. Epidermal cells of *Doodia kunthiana*. A_1 Upper epidermis, A_2 Lower epidermis. B. Epidermal cells of *D. lyoni*. B_1 Upper epidermis, B_2 Lower epidermis. C. Rhizome scale of *D. lyoni*. D. Rhizome scale of *D. kunthiana*. E_1 1–4. Pinnae of *D. kunthiana*. F_1 1–4. Pinnae of *D. lyoni* (4 the same as 3 but with indusia omitted to show venation pattern).

sometimes without a sorus. The distance of the fertile vein from the midrib governs the position of the sorus and this relationship is also among the characters which have previously been used in contrasting the two species. *D. kunthiana,* on the one hand, normally bears fertile veins rather distant from the midrib, often occurring one-half or more of the distance to the margin. *D. lyoni,* in contrast, typically bears fertile veins in close proximity to the midrib, these veins frequently arising approximately one-third the distance to the margin. This nearness to the midrib results frequently in a sorus which at least partially covers the midrib. However, the sorus in some *D. lyoni* fronds may be further removed from the midrib than one-third of the distance to the margin, as Skottsberg has pointed out.

Another portion of the pinna from which distinctions of the two species may be made is the indusium. Macroscopically, the shape of the indusium frequently gives a clue as to the identity of the fern. Those of *D. kunthiana* (fig. 3, C, D) are typically oval or roughly circular in outline and possess regular, unbroken margins. They are, without exception, discrete units, never overlapping with adjacent indusia. On the contrary, the indusium of *D. lyoni* (fig. 3, A, B) is, as a rule, much more elongate with reference to an axis parallel to the midrib so that adjacent indusia may overlap. The free margin of the indusium is frequently irregular in outline and may partially or completely cover the midrib. Microscopically, two striking differences distinguish the species in most cases: the size of cells composing the indusium appears to vary directly with the size of the indusium. Thus the small indusium of *D. kunthiana,* which is otherwise morphologically the larger-sized of the two species, is composed of relatively small cells while the large indusium of *D. lyoni,* with its tendency toward elongateness, displays generally larger indusial cells. Some plants of *D. lyoni* with small indusia, however, may grade into the range of *D. kunthiana* as far as cell size is concerned. The distribution of hairs on the indusium has been mentioned above in connection with the hairs. However, it might be well to mention here that in most of the collections of *D. kunthiana* the hairs (of both types) are very scant on the indusium if not entirely lacking, while in the collections of *D. lyoni,* indusia exhibit an abundance of linear hairs and rarely some isolated capitate hairs.

The rhizome scales do not appear to offer any definite grounds for distinguishing species. They range in color in both species from a dark brown to a rich black. Those of *D. lyoni* (fig. 4, C) are smaller and clear readily in NaOH. Scales of *D. kunthiana* (fig. 4, D), on the other hand, are larger in size and are noticeably more resistant to clearing action.

The present study, both quantitative and anatomical, has brought out some important criteria for differentiation of *Doodia kunthiana* and *D. lyoni.* It might be advantageous, therefore, to bring together, at this point, this new information in the form of a detailed description of *D. lyoni.*

Description. A fern of small to moderate size, its habitat restricted to vertical rocks along stream banks; the fronds tufted, 7–20 photosynthetic ones per rhizome, with several persistent dead stipes in addition; rhizome suberect, short, with numerous long roots, up to 6.5 cm. long; rhizome scales elongate-lanceolate, up to 6 mm. long, up to 1 mm. broad at base, densely crowded at tip of rhizome, brown to almost black, the scale margins with a few shallow lateral emergences, the central cells with thicker and darker lateral walls (fig. 4, C); fronds once pinnate at base becoming pinnatifid and finally confluent at the apex; stipe and rachis deeply ridged above, black at base, becoming brown to straw-colored distally, the younger rachises clothed with hairs which fall off at maturity; stipes 0.5–1.8 mm. in diameter, 0.7–9.5 cm. long; blade 5.2–23.5 cm. long, 0.9–3.9 cm. wide; segments 11–30 pairs, the basal 1–2 pairs being distinct and separate pinnae and possessing stalks, the central segments sessile, the distal segments being merely increasingly shallow lobes; pinna margins regularly dentate, provided with linear hairs; lower epidermis bearing scattered linear hairs comprising 1–2 cells along veins, capitate hairs of 2–3 cells infrequent; veins anastomosing to form a row of areolae on each side of rachis, infrequently a second rank of isolated and irregularly scattered areoles present, free veins terminating just short of sinuses along dentate margin; sori 5–22 per pinna, each sorus arising on a fertile vein formed by an anastomosis of adjacent veins, usually in close proximity to the midrib; the indusium elongated along midrib, frequently overlapping with adjacent indusia to hide the midrib, clothed with numerous linear hairs; individual indusial cells relatively large, elongate in outline, 170–250 μ in average greatest dimension; sporangial capsules between 290–360 μ before dehiscence; annulus with 21 (15–23) thickened cells; spores light-brown, smooth-walled, translucent, elliptic, 35–45 μ long, 27–32 μ broad.

SUMMARY

A reappraisal of the validity of *Doodia lyoni* Degener as a species is deemed necessary because it has seemed possible that it might be only a variety or form of *D. kunthiana*. Therefore, certain quantitative and anatomical studies have been carried out with *D. kunthiana* and *D. lyoni* and the following points of distinction between them have been brought to light:

1. Ecologically, *D. lyoni* is much more restricted and rarer than *D. kunthiana*, which, while existing in the same type of habitat as and together with *D. lyoni*, also may occur in a number of other situations.

2. The size alone of the entities involved, though generally indicative of the species, should not be relied on exclusively.

3. The stipe-blade ratio is a valuable species indicator although there is a slight overlap in range.

4. The number of stalked pinna pairs present is fairly consistent, *D. lyoni* being limited to 1–2 pairs, and *D. kunthiana* usually to 3–5.

5. The type and distribution of hairs on the abaxial laminar surface is of the most value in identifying the species.

a. Predominance of *capitate hairs* or absence of hairs of any type is

characteristic of *D. kunthiana,* while a predominance of *linear hairs* concentrated especially on the indusium is characteristic of *D. lyoni.*

b. *D. lyoni* possesses marginal hairs while *D. kunthiana* lacks marginal hairs.

6. Position and shape of the sorus is frequently valuable in identification. Sori of *D. kunthiana* always remain as discrete units, and are, for the most part, remote from the midrib. Those of *D. lyoni* tend toward elongation along the midrib, and are frequently overlapping with adjacent sori. Being closer to the midrib the sori of *D. lyoni* may partially or completely cover it.

7. The indusial cells of *D. lyoni* tend to be larger than those of *D. kunthiana.*

8. The above evidence indicates some clear discontinuities in the characters studied and these differences are considered important enough, *taken together,* to allow maintaining taxonomically a species status for *Doodia lyoni.*

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TORREYA

TETRAD ANALYSIS THROUGH SECTIONED MODELS*

CLARA S. HIRES

A minute object must be difficult to see clearly if scientists contradict one another in describing it. Such controversy surrounds the mother-cell and its division into spores or pollen. Contradictory interpretations of fern tetrads inspired this study.

The baffling particle must be mounted and highly magnified for detailed microscopic exploration. Variations in mounting methods and lighting create differences of opinion. Frequently only one focal level from one angle is seen. Even when the object can be examined from top to bottom, degree of elevation and depression of contours are difficult to judge. All differences must be reconciled to determine correctly each characteristic 3-dimensional form.

Everyone agrees that a spherical spore mother-cell normally divides to form a tetrad of four identical spores. Also, it is evident that each spore has to begin with one curved area, the side on the outer surface of the mother-cell. But what is the tetrad arrangement within the mother cell? Do all four spores meet at the central point, or do they lie in pairs on long axes through the center?

If the spores of the tetrad meet at a central point and have three flat sides edged by three scars or triradiate ridges, they are said to be tetrahedral (trilete). If they look like quarter sections of a rounded apple with two flat sides and one scar, they are called bilateral (monolete). The pairs of monolete spores may lie with their axes running in one direction along a single line, or with their axes running in two directions at right angles.

To determine the method of division, microscopists may section developing tetrads. They make microtome sections across mother-cells and examine patterns of walls, nuclei, and products of nuclear division seen within the interior. More frequently they stain the sections for chromosomes than for wall structures. This overlooks changes produced by staining and mounting methods and use of the microscope, which also must be considered later. What seems like common-sense analysis supported by tradition can be deceptive unless the relation of the section to the whole tetrad is clear, as may be indicated by comparison with large, sectioned models.

We have tried to show how whole tetrads would appear if large enough to be handled manually, turned over, and then cut through in a series of planes. To do this, we made plasticene models of typical trilete and monolete spherical tetrads as if magnified 2000–3000 diameters. The spheres were approximately 2″ in diameter, with a nucleus ¾″ in diameter centrally located in each of the four spores.

These rounded models were cut across at various angles as a mother-cell might be cut with a microtome. First we cut to show generally accepted wall patterns, then sectioned at angles to demonstrate unusual wall arrangements.

The variations in geometric patterns shown by the interior walls on the cut plasticene sections surprised us. Several unusual line arrangements were found which had not been anticipated. Similar designs were found in both trilete and monolete sections, arrangements previously assumed to indicate one or the other type. Some of these were a single line, a line branched at one or two ends, and a cross.

As further checks, we also made transparent plastic models of tetrads, showing positions of walls and nuclei.

The accompanying figures show unsectioned and sectioned tetrad models. Trilete and both types of monolete wall arrangement are illustrated. A picture at one end of

* Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

each row shows a completely assembled tetrad put together from the series of sections depicted beside it.

The sections show the arrangement of walls at various levels, and suggest how the nuclei might appear if resting in the center of each spore. The same nucleus would appear to vary in size, being smaller at top or bottom focus than through the center. It might be completely out of focus if the section was not cut to pass through it. A slight change in cutting slope might change a straight line to a bent line.

Other patterns will be discovered. These are just a few that help us check our interpretations as well as those of others. Since we know of no other series of this type, we believe these may be of value to others studying tetrad division and spores.

I am most grateful to those who have challenged my opinions as well as to those who have helped me produce my checks and double checks. No one method of study alone can give the correct answer to the problem of spore arrangement in the tetrad; but I hope this approach will be found useful, at least interesting, to others.

Maud Mary Griswold, who constructed the models, described our methods at a fern spore symposium at Mistaire Laboratories on June 4, 1955. At that time, we demonstrated our methods so that others can duplicate our studies. We invite your reactions and questions.

Mistaire Laboratories
152 Glen Avenue
Millburn, New Jersey

Geometric Patterns Within Tetrads Revealed By Models

To aid microscopic interpretation of spore tetrads and microtome sections, models —2″ in diameter—of spherical tetrads were constructed for comparison of three types: (Note numbers on plates.)

Monolete
- 1-directional— (M-A) four spores like quarter sections of round apple, arranged in pairs along central axis (M1-6),
- 2-directional— (M-B) similar-shaped spores, arranged in pairs with axes at right angles (M7-12),

Trilete— all four spores meet at central point (T1-10).

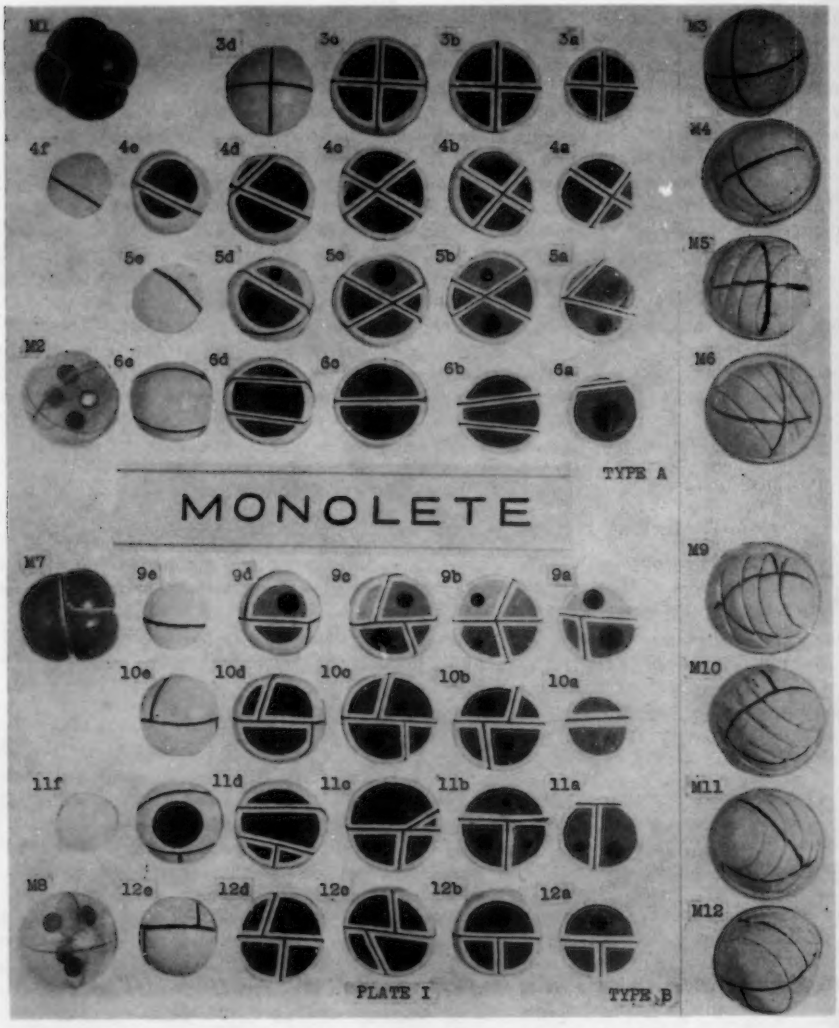
Unsectioned tetrads of plasticene (M1, M7, T1 and T3) and transparent plastic (M2, M8, T2) are shown. Other models (M3-6, M9-12, and T4-10) were sectioned to demonstrate wall and nucleus patterns. The individual sections of each tetrad were labelled consecutively with small letters (M3a-d, M4a-f, M5a-e, M6a-e, M9a-e, M10a-e, M11a-f, M12a-e, T4a-e, T5a-f, T6a-g, T7a-h, T8a-f, T9a-f, T10a-f). Some were cut in parallel series, others at angles to show more unusual arrangements. A round nucleus, ¾″ in diameter, was located in the center of each spore.

In the accompanying plates, figures are reduced from 2 inch models. The series were photographed, and the assembled tetrads were photographed—each of the latter shown in the plates beside Section "a" of its series.

Black lines on the models indicate lines of spore contact. White lines and areas are spore walls. Grayish lines on assembled models are sectioning cuts. Nuclei, when visible, appear as round dark areas, large when cut through their centers, smaller when cut above or below center.

The accompanying chart compares similar patterns of walls and nuclei in three types of tetrads. A slight variation in cutting would suggest other possibilites. It is important to consider *both* wall and nucleus arrangements in analyzing a single cut section.

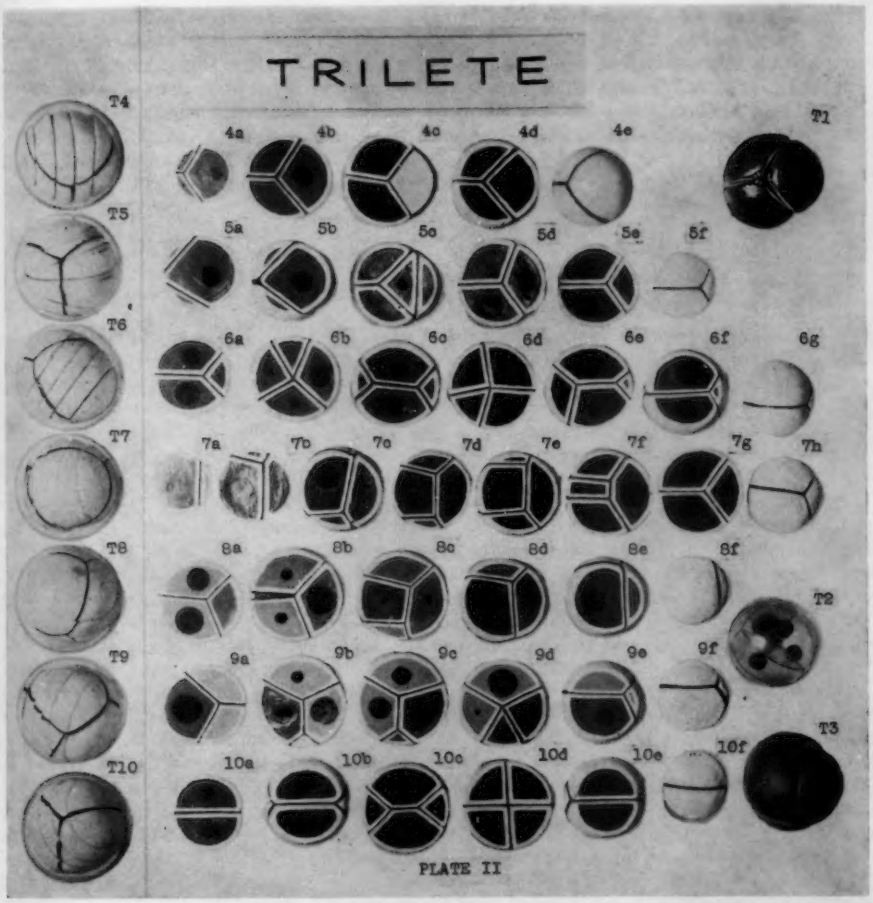
Summary Of Tetrad Patterns For Comparison	*Plate Nos.* M-Monolete I types A & B T-Trilete II
M-A (One-Directional Monolete—Figs. M1 to M6)—all typical.	
1. One line—2 spores—0 to 2 nuclei, all sizes—in series to tetrad center.	M4e,f, M5e M6a,c



2. Two lines, parallel or slightly converging—3 spores—0 or 1 tiny or large nuclei.	M6b,d,e
3. Two lines, joined as "V"—triangle effect with outer wall—3 spores—0 to 2 nuclei of all sizes.	M4d, M5a
4. Right-angle cross—4 spores—0 or 4 nuclei—cross repeated through tetrad.	M1, M3, M5 M3a,b,c,d
5. Unequal cross—4 spores—0 to 2 unequal nuclei—spore areas unequal.	M4, M4a,b,c M5b,c

M-B (*Two-Directional Monolete—Figs. M7 to M12*)

1. One line—2 spores—as in M-A.	M9e, M10a
2. Two lines, parallel or converging, with tiny side branch.	M11d,e (effect)
3. Two lines—"V" with triangle effect—as in M-A.	M10e, M12e (effects)



4. Right-angle cross—4 spores with 4 nuclei in unequal pairs—central section.	M9b (effect)
5. Unequal cross—2 pairs of spores with 2 similar nuclei, or 4 unequal spores with 2 unequal nuclei—nuclei in larger areas central section.	M9b, M11c
6. One straight line, once-branched—3 spores—1 to 3 nuclei (1 larger than others)—easily confused with trilete.	M9a, M11a,b M12a,b
7. One straight line, twice-branched—4 spores—0 to 3 nuclei.	M9c,d, M10b,c M10d, M12c,d,e
8. Triradiate lines lacking, similar effects might be confused.	M9a (effect)

T—(*Trilete—Figs. T1 to T10*)

1. One line—2 spores—outer sections—0 to 2 tiny nuclei.	T7a, T8f T10a,f
2. Two lines—"V" with triangle effect—3 spores—1 large nucleus.	T5a,b, T8e, T7
3. Equilateral triangle of 3 straight lines with 1 small or large central nucleus—3 other spores edge triangle—in trilete only.	T5c, T7 (effect)
4. Right-angle cross—4 spores—2 small nuclei—central section only.	T10d

5. Unequal cross—4 spores—0 to 3 large or small nuclei—central section only.	T6b,d T8b T9d
6. One straight line, once-forked near tetrad edge—easily confused with a straight line.	T6g, T7b, T9e,f (effects)
7. One straight line, twice forked,—4 spores—0 to 3 nuclei. May give square to oblong effect with outer tetrad wall.	T3, T6c,e, T7d,e,f, T8c T10b,c,e
8. Triradiate lines from 1 point, not at right angles—3 spores—0 to 3 nuclei.	T1,4,5,10 T4a,b,c,d,e T5d,e, T7g T8a, T9a

John E. Potzger

Dr. John E. Potzger was born in the "limber country" of Presque Isle, Michigan, on July 31, 1886. From his earliest childhood he loved the unrestricted life of the wilderness, and much time was spent in the woods. The wilderness imparted to him that intangible something called TENACITY, which was a valuable ally throughout his life.

After attending the Lutheran Teachers College from 1901 to 1906, he devoted 24 years to teaching in a Lutheran Day School in Indianapolis, Indiana, where he was organist, choir director, and leader of young people. During this time, several years of tremendous effort were devoted to musical training, which finally ended with studies under the Belgian artist, Leon Sampaix, at the Metropolitan School of Music in Indianapolis, Indiana, and at the Ithaca Conservatory of Music at Ithaca, New York.

There followed a period of years which Dr. Potzger described as the "educational grist mill period" during which education in general appealed. Into this period were crowded night classes at Butler and Indiana extension in an English-German major, charcoal drawing classes at the Herron Art Institute, a summer's work at Chicago University in education, and in 1925 he entered Butler University. It was here that he came under the influence of the late Dr. Friesner and discovered that a major in botany would answer more of his questions about the world in which he lived than an English-German major, and could give him more personal enjoyment from the outdoors.

Thus began the most dynamic work in botany, which ended in an A.B. at Butler in 1927 and an M.A. in 1931, all work completed in summer school and night classes after a full day of teaching. In 1931 he resigned from his teaching and entered Indiana University to work toward a doctorate in ecology, which he received in 1932. It was during this period that he came under the influence of Dr. Frank C. Gates at the Michigan Biological Station and Dr. S. A. Cain at Indiana University. It was also during this period that he realized the complex nature of vegetation as shown in the sensitively-balanced control of oak-hickory and mixed hardwoods in Indiana. Out of his thesis came the realization of that sensitive balance, that oak-hickory is not climax in Indiana, and that mixed hardwoods always control the vegetation where soil factors do not interfere with the full value of normal rainfall. Out of that thesis also came the burning desire to study this condition in greater detail "on miles and miles of quadrats."

In 1932 he entered Butler University as instructor in Botany and in 1952 assumed the headship of that Department. Research was one of his activities from the day he returned to Butler, with an early period that moved in taxonomy, quadrat studies, ring counts, morphology, and pollen analysis. Taxonomy was finally limited to the grasses of Indiana, forest sociology was confined to specific comparatively undisturbed areas, and pollen analysis became of prime importance.

While he was always active in research, he never forgot the admonition which Julius Sachs gave his followers at parting: "Gentlemen, remember, first of all you are teachers." In an effort to extend scientific information to junior high school students, scouts, etc., he prepared two small books on trees, three on birds, and jointly with his wife one on insects. Throughout these many years he thus had the satisfaction of teaching from 45,000 to 65,000 potential biologists every year.

Since 1940 Dr. Potzger's work with pollen analysis has covered wide geographical

areas. For the last four summers he has gone to Mont Tremblant biological station to conduct studies in forest history. His studies of the post-glacial period proved that Canadian spruce once covered Eastern North America. Perhaps his most significant contributions to science were the discovery of spruce and fir pollens in Texas bogs, the apparent cooling due to local glaciation throughout southern Quebec, and the theory that southern New Jersey served as a refugium during Pleistocene times in which the post-Pleistocene northward migration of forests originated.

John Ernest Potzger July 31, 1886—September 18, 1955.

His membership in scientific organizations totaled ten, but his main interests were the Ecological Society of America and the Central Association of Science and Mathematics Teachers. It was a greatly appreciated honor when these organizations, one in 1948 and the other in 1953, elected him to the presidency.

Among the privileges enjoyed were research grants from the American Philosophical Society for work on Isle Royale (1948), in the Quetico-Superior Forest (1950) under the appointment of the President's Committee on the Quetico-Superior Memorial Forest, research at the Trout Lake Biological Station of the University of Wisconsin in 1940, and the honor to be appointed a member of the research team at the Biological Station at Mont Tremblant Park, Quebec, in '52, 53, 54.

Dr. Potzger was a stimulating, energetic, and modest man. With all his activities he was never in a hurry, and was always ready and willing not merely to help, but to do more than his share and to give unsparingly of himself. Whether or not his efforts of the past 25 years will be a contribution to ecology and to the understanding of climate and vegetational changes since glacial times, or whether he was an ecologist with penetrating thought

will no doubt be determined by another generation. However, through his research, Indiana vegetation and its post-glacial history will be better understood.—ERNEST S. HAMILTON, Department of Botany, Rutgers University.

FIELD TRIP REPORTS

Sept. 3. Richmond Valley, Staten Island, N. Y. The group walked through brushland, woodland and a private estate to the beach, and back along a country road. Typical fall flowers were seen and many trees and shrubs in fruit, among them *Sassafras, Viburnum dentatum, V. prunifolium,* and *V. acerifolium;* a Chinese chestnut with burrs on the ground as well as on the tree; a Persimmon with some fruit already ripe. The oaks *Quercus rubra, Q. alba, Q. phellos, Q. palustris, Q. velutina, Q. marilandica, Q. Prinus,* and the hybrid *Q. Rudkini* = *X phellos* and *marilandica* were studied, all in fruit.

A stand of the morning glory, old man of the earth, *Ipomoea panäurata* was also visited. Attendance 12. Leader, Mathilde P. Weingartner.

Sept. 10. Pine Barrens of New Jersey. Joint trip with the American Fern Society. Botanizing was done at Atsion, Hampton Park, Wading River and Oceanville. Some 185 species of pine barren plants were observed. Of special interest were *Gentiana autumnalis* (in flower); the large station of *Lygodium palmatum; Phoradendron florescens;* a well-fruited *Castanea dentata* and the fine bog containing large quantities of *Lycopodium carolinianum* and many plants of *Dionoea muscipula* (brought from North Carolina in 1948 and increasing here ever since.) Attendance 26. Leader, L. E. Hand.

BOOK REVIEWS

Geography of the Northlands. By G. H. T. Kimble and Dorothy Good, American Geographical Society and John Wiley & Sons, Inc., New York and London. 534 pp. (American Geographical Society Special Publication No. 32) 1955. $10.50.

An over-all treatment of the circumpolar Arctic and Subarctic regions is indeed a welcome addition to geographical literature. This fine volume, prepared by fifteen contributors under the able editorship of George Kimble and Dorothy Good will be constantly useful both to those who deal with the Northlands and to those whose interests include a broad appreciation of the world as a whole.

The book treats the main features of the North topically in the 13 chapters of the section entitled Systematic Studies; and then, in the section called Regional Studies, devotes a chapter each to the fourteen main geographical areas. A large fold-in map is provided showing the main political subdivisions, cities and towns, physiographic features, and the delimitation of Arctic and Subarctic adopted in the volume. Many smaller maps, as well as excellent photographs and diagrams illustrate the chapters. Short bibliographies are provided with all of the chapters. Statistics are used adequately but not oppressively, and the writing is generally very readable, though, of course, uneven from chapter to chapter. Most of the chapters hold the ordinary reader's interest very well, though several of them approach dullness. The book will unquestionably find great use as a college text in geography.

For the present audience the portions of the book dealing with biogeography are, naturally, of greatest interest. The editors were fortunate, indeed, to have the chapter on Biogeography of the Land and the Inland Waters written by Pierre Dansereau. In his usual brilliant style he has taken in this enormous subject, digested and generalized it down to a 46-page treatment which is well-organized, clearly written, illustrated by photographs and diagrams, and which gives a fairly satisfying regional picture. Specialists on restricted areas and particular features will naturally find his treatment inadequate and will complain that their interests have been neglected. From the broad viewpoint, though, there could have been little improvment. The first few pages, in fact, may be recom-

mended to all beginning vegetation students and others interested as an excellent introductory presentation of the basic nature of vegetation and the philosophy of its study. One might quibble somewhat on a few points of terminology. On page 94 and elsewhere the word "heath" is used in two entirely different senses, applying to a type of plants and to a type of vegetation, in a way which may be confusing. And it seems, to the reviewer, quite unfortunate that the term *taiga* is used for the lichen woodland rather than in the original and better understood sense of the northern coniferous forest, here termed *boreal forest*. When folk terms are used for vegetation formations it would appear better to adhere to the original meaning unless another meaning has become so universally adopted that no confusion will result. In this book even the editor points out that Dansereau's use of *taiga* differs from that of many authorities, and in later chapters of the book, notably those on the Russian regions, the word is applied in its original sense, to coniferous forests. The strength of the present chapter is that it effectively presents the biotic communities of the Northland as functioning dynamic entities, with due regard given to both plants and animals, including man.

The botanical interest in this book is not confined to the chapter discussed above, however. The chapter on Marine Life, by Maxwell J. Dunbar, is also an able bioecological presentation, emphasizing the role of green plants in the productivity of the marine environment. The chapter on Renewable Resources gives a great deal of attention to the subarctic forests and to the animals which depend on the plants for food and shelter. Each regional chapter has some description of the vegetation. These treatments vary greatly in completeness, approach, and viewpoint. They vary from a bare mention of the subject in the chapter on Alaska to very interesting short discussions in the chapters on Scandinavia, Svalbard, and especially, the Russian regions. It might have been a good idea to have had someone more familiar with biology and ecology scan these regional accounts, as well as the topical chapters, and make suggestions to the various authors. More adequate treatments might have resulted in some cases and small errors would have been eliminated.

On page 440 there is the very interesting statement that "To shelter rare species of plants and animals, and to preserve the untouched primitive landscape of the taiga, a reservation has been created in western Siberia in the Konda-Sos'va region, covering an area larger than 2,000 sq. mi."—F. R. Fosberg, Falls Church, Va.

Insects of Micronesia. Volume 1. Introduction. By J. Linsley Gressitt B. P. Bishop Museum, 1954. 257 pages. $3.50.

After Insects of the Society Islands, of the Marquesas and of Guam, the Bishop Museum, with help from the National Science Foundation, has now embarked on publication of an ambitious series: Insects of Micronesia. Of this, volume I, Introduction, volume 2, Bibliography, and various parts of the following volumes have been published. The project is supervised and coordinated by Dr. J. Linsley Gressitt, who is the author of the introductory volume. Dr. Gressitt has persuaded more than 100 specialists all over the world to study the collections of insects and other small land arthropods from Micronesia and to collaborate in the published series. This volume is intended to give them the necessary background knowledge of the area and up-to-date evaluation of insect collections.

Micronesia, thus named by Admiral Dumont d'Urville in 1832, comprises a number of archipelagoes of the tropical Pacific. The main ones constitute the former Japanese Mandate, now U. S. Trust Territory of the Pacific Islands: Marianas, Caroline and Marshall groups, with the addition of a few scattered islands. The region in the Insects of Micronesia series is considered to include, in addition to these, the Gilbert Islands (a British colony) and the Volcano and Bonin Islands now under provisional U. S. government. Because of its stormy history and the small size of even the larger islands, Micronesia was, until recently one of the less-well-known regions of the Pacific. Since the end of World War II, a great many studies in the geography, anthropology and natural sciences of these islands have been carried out, and their results are slowly trickling into

the literature. The modest title chosen by the Bishop Museum hides effectively the fact that Dr. Gressitt's introduction includes the first comprehensive geographic and natural history description of Micronesia. As such, this volume will be indispensable to anyone interested in the Pacific Islands. The description of the area includes general considerations on the geology, soils, land flora and vegetation and land fauna, accounts of the climates of the different island groups, and descriptions of the individual islands. The section on the flora is based on the Enumeration prepared by Kanehira in 1935, so does not take into account the results (mostly still unpublished) of 20 years active collecting since then. The vegetation section is largely adopted from Fosberg's unpublished account (prepared in 1946) in the Economic Survey of Micronesia report, but is well illustrated by photos. The following two parts, although more specifically entomological, also contain much information of interest to a wider audience. They are devoted to ecology, where insects are studied in relation to the natural setting and to other living organisms, especially plants, and to economic entomology. The next part is really the only one of more restricted interest: it is an account of the recent collections made in Micronesia, with data on the authors and main collection localities. The specialists for whom this volume was prepared have received, from all these collections, the animals belonging to the groups they are studying, and thus will have all the necessary information pertaining to the specimens.

The volume concludes with a general bibliography, which will usefully complete the entomological bibliography in volume 2, a gazetteer of place names, cross-indexed for synonyms, and a very complete index to the whole volume.

It always takes much courage to write a monographic treatment of any subject while it is being intensively studied. The author has the frustrating experience of feeling his work getting out of date as fast as he can advance it. This is partly true of this Introduction. However, the description of the natural history of Micronesia, in a solid geographical frame, was a much needed basis for all further research. Dr. Gressitt has utilized the most recent literature and ferreted out much unpublished information. It will take a great many years before all recent and current research is available in published form and can be utilized to expand and correct the present Introduction. Meanwhile, this will be a most useful manual for all workers in the area. Dr. Gressitt is to be thanked and congratulated on carrying out so well such a task, and the Bishop Museum must be commended for the excellent presentation, and especially for the numerous maps and well-reproduced photographs.—MARIE-HÉLÈNE SACHET, Washington D. C.

Biological Specificity and Growth. Eidited by Elmer G. Butler, Princeton University Press, 1955. $5.00.

This book includes eleven of the twelve papers presented at the Twelfth Growth Symposium held at the University of New Hampshire in June, 1953, sponsored jointly by the Society for the Study of Development and Growth and the Committee on Developmental Biology of the National Research Council. The report by Michael Heidelberger on the "Relationship between chemical structure and immunological specificity" is, unfortunately, not included.

It is a difficult thing to summarize briefly the content and significance of these excellent reports or to gain from the printed chapters the spirit of critical but friendly give-and-take which prevailed at the meeting.

The following active investigators have written chapters on the topics as indicated:

(1) Ontogeny of the blood and related antigens and their significance for the theory of differentiation, by A. M. Schechtman.
(2) Immunobiological approach to some problems of induction and differentiation, by M. W. Woerdeman.
(3) Immunogenetics, by M. R. Irwin.
(4) Enzyme development as ontogeny of specific proteins, by S. C. Shen.
(5) Cellular differentiation and the development of enzyme protein in plants, by R. Brown and E. Robinson.

(6) Some problems of specificity in the sexuality of plants, by John R. Raper.
(7) General aspects of immunological reactions with bacteria and protozoa, by James A. Harrison.
(8) Specificity in the relationship between host and animal parasites, by W. H. Taliaferro.
(9) Compatibility and non-compatibility in tissue transplantation, by Harry S. N. Greene.
(10) Specificity in growth control, by Paul Weiss.
(11) Specificity of nuclear function in embryonic development, by Robert Briggs and Thomas J. King.

The breadth of treatment of the subjects varies considerably. Dr. Greene reports almost entirely on the experiments of himself and associates (26 of 28 references) while Dr. Scheehtman gives a more general survey of his topic including only seven references to the work of himself and associates out of a total of 110. Altogether the chapters do present in their various ways significant aspects of the great topic of biological specificity as related to growth processes, and the book is a necessity for those working in this field of study and for those having a general interest in problems of biological specificity. It is regretted by the reviewer that the masterful summary of the conference given at the close of the meetings by Dr. Paul Weiss was not included.—ALAN BOYDEN, Rutgers University.

Genetics and Metabolism. By Robert P. Wagner and Herschel K. Mitchell. John Wiley & Sons, New York, and Chapman & Hall, Limited, London. 1955.

A book of the character of that of Wagner and Mitchell has been needed for some time. More and more are the steps of metabolic processes understood in terms of chemical reactions. Instead of genes influencing an end group or a terminal series of reactions (syndrome), which heretofore has been the only distinguishable evidence of gene action, it is now possible to pin-point their actions to a particular part of a process and delineate other factors to the subservient position of results of the disturbed metabolism. While both the authors are biologists, one of them is mainly trained as a chemist. Each one contributes to the book in terms of his own specialty. The book contains a remarkable amount of material in its relatively small size (400 pages), clearly written but requiring real study to get the most out of it. Geneticists generally may not be well-grounded in chemistry. Perhaps even more so the average chemist may not be trained in genetics. The main criticism of this reviewer is the general paucity of explanation of technical terms. The book combines two highly specialized departments of science, the one to help explain the other. The authors realize this and suggest consultation of other references. The choice of such references is very hard for one somewhat unfamiliar with the field to make. Even at the expense of some extra length of the context, the reader would more quickly grasp the intent of the authors if this were done. The addition of an adequate glossary might be sufficient. The many charts and schematic summaries of complicated metabolic processes and chemical reactions are excellent. Certainly the geneticist would do well to have this book on his shelf and consult it often if he has any desire to do more than describe the gross appearance of hereditary traits. The same applies to the chemist who may be interested in what determines the characteristics of any organism.—M. G. BOYDEN, Rutgers University.

Field Book of American Wild Flowers. By F. Schuyler Mathews'. Completely revised and enlarged by Norman Taylor. xxix + 601 pages, 29 color plates, about 1000 figures. G. P. Putnam's Sons. 1955. $5.00.

The text is completely reset and the boxed in names with flower colors and blooming periods have been placed at the beginning of the paragraphs. The color plates are renumbered and that of the Rose has been omitted. The Cashew, Buckthorn and Vine

Families are left out and the Crowberry and Cactus Families added. The text is largely identical with the previous edition but numerous changes are made and a few additional figures included by Eduardo Salgado.—P. L. RICKER, The WildFlower Preservation Society.

Garden Spice & Wild Pot-herbs. By W. C. Muenscher and M. A. Rice with woodcuts by Elfriede Abbe. Cornell University Press, Ithaca, New York. x + 211 pp. 56 pl. figs. 1955. $5.75.

Though there are numerous books dealing with garden herbs and edible wild plants, probably none of them is as useful as this volume. Most such books treat the uses of the plants but are of little or no use in identifying them. Here, at last, is a book in which most of the spice herbs and wild pot-herbs are beautifully illustrated with detailed, accurate and highly artistic woodcuts by one of the foremost botanical artists. In addition, the text contains good descriptions and detailed discussions of the uses of the plants. In many cases recipes are given.

The text is divided into four parts. Part one contains the descriptions, illustrations and uses of herbs. Part two treats their propagation and methods of harvesting, drying and storing. Part three deals with the wild pot-herbs and part four contains a discussion of classification and naming of plants. In the back of the book is a large plate of illustrations of seeds, a glossary of terms and a very inclusive index.

The volume is a must for all persons interested in herbs and for horticultural libraries. Aside from its usefulness the book is beautifully printed and bound. These facts together with the exceptional woodcuts would make it attractive to any collector of unusual books.—DONALD G. HUTTLESTON, Brooklyn Botanic Garden.

Drawings of British Plants. By Stella Ross-Craig. Part VIII. Rosaceae (1). pl. *1–40*. index. 22 S 1955. London: G. Bell and Sons Ltd.

The current installment of these fine drawings (see also Bull. Torrey Club 80: 232) includes the British species of *Prunus* and *Rubus*, with *Potentilla* and related genera. Of the more than 300 species of *Rubus* lately recognized in Great Britain, 17 were chosen for illustrations. A prefatory note explains the basis of choice; largely the opportunity to secure flowers and fruits from the same individuals—a commendable step towards greater accuracy in diagnosis. These are some of the best pen-and-ink drawings I have seen for a long time; they seem to improve as they go on. At the modest price (just over a dollar) they are a bargain.—H. W. RICKETT.

The Genus Clarkia. By Harlan Lewis and Margaret Ensign Lewis. University of California Publications in Botany 20: 241-392. 1955. University of California Press, Berkeley and Los Angeles. $2.00.

In this monograph the genus *Clarkia* (Onagraceae) comprises 33 species, all but one limited to western North America. The genus is closely related to but clearly distinct from *Oenothera*. Like the latter genus, *Clarkia* has been subjected to considerable genetic and cytological study and the monograph is particularly timely for this reason. Although the authors state that the investigation is not yet complete it is, by almost any standard, a most complete piece of work. All the North American species have been studied in the field; progenies of all the species have been grown in the experimental garden; chromosome numbers have been determined for all taxa, generally from several plants; "several thousand" interspecific pollinations have been made involving 202 species combinations; and more than 10,000 herbarium specimens were examined. Fortunately, the writers have not found it necessary to cite all these but have for the most part contented themselves with listing representative material, although even here the citations may occupy more than a page.

According to the authors "the most important criterion to delimit the various taxa and to determine their relationships has been morphology. . . ." However, it is clear that hybrid sterility has played a role in the separation of *C. amoena* from *C. rubicunda* and *C. bottae* from *C. cylindrica*. It is also interesting to note that *C. biloba brandegeae* more closely resembles *C. dudleyana* than it does a sister subspecies, and that *C. lingulata* is separated from *C. biloba australis* only in shape of petal and chromosome number. On the other hand, several species include races which are intersterile or nearly so and these are not given recognition as species because of the lack of obvious morphological differentiation. One species, *C. purpurea*, is frankly recognized as a waste basket and virtually "represents the residue after the other demonstrably valid species of the Section Godetia have been set aside." These comments are not by way of criticism but rather to show the complexities of monographic work when all lines of evidence are taken into consideration.

The thirty-three species, nine of which have been described at one time or another by Lewis and his wife or students, are grouped into 10 sections. Subspecies are recognized in many of them. These are geographical except in *C. purpurea* where artificial subspecies are employed to designate "the most conspicuous morphological expressions" of the species. There is a worthwhile discussion of variation within the various taxa, although scant attention is paid to the cultivated strains, and one variant is designated as a form. In addition to the distribution maps and very complete keys, there are a number of line drawings which should facilitate identification. The phylogenetic chart and the accompanying discussion are excellent. Since most of the allopolyploid species have now been artificially synthesized and detailed chromosome studies have been made, one is inclined to have considerable faith in the conclusions regarding phylogeny. It is gratifying to find an index to species and to exsiccatae, although the latter perhaps will be used only by curators of herbaria. All in all then, this monograph can be considered an outstanding work, in which the authors face and solve many of the problems involved in modern work in taxonomy.—CHARLES B. HEISER, JR., Indiana University.

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LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITORS OF THE TAXONOMIC INDEX

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